Ubiquitin - Related Research Products and Services

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E1 Activating  E2 Conjugating  E3 Ligase  Deconjugating  Ubiquitin  SUMO  NEDD8  ISG15  UFM1
Autophagy  FAT10  Small Molecule Inhibitors  Antibodies  Kits  Substrate Proteins  Affinity Matrices
The Ubiquitin Proteasome Pathway (UPP) is the principal mechanism for protein catabolism in the mammalian cytosol and nucleus. The highly regulated UPP affects a wide variety of cellular processes and substrates and defects in the system can result in the pathogenesis of several important human diseases. The central role of the UPP in biology has been recognized with the Nobel Prize for Chemistry which was awarded to Avram Hershko, Aaron Ciechanover and Irwin Rose in 2004. The UPP is central to the regulation of almost all core and the 19S regulator (Degradation). This classical function of Degradation of a protein via the Ubiquitin Proteasome Pathway (UPP) involves two discrete and successive steps: tagging of the substrate protein by the covalent attachment of multiple ubiquitin molecules (Conjugation); and the subsequent degradation of the tagged protein by the 26S proteasome, composed of the catalytic 20S core and the 19S regulator (Degradation). This classical function of ubiquitin is associated with housekeeping functions, regulation of protein turnover and antigen-peptide generation.

More recently, it has become evident that protein modification by ubiquitin also has unconventional (non-degradative) functions such as the regulation of DNA repair and endocytosis. These non-traditional functions are dictated by the number of ubiquitin units attached to proteins (mono versus poly-ubiquitination) and also by the type of ubiquitin chain linkage that is present.

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Conjugation
Ubiquitin becomes covalently linked to itself and/or other proteins either as a single molecule or as poly-ubiquitin chains. The attachment of ubiquitin to the ε-amine of lysine residues of target proteins requires a series of ATP-dependent enzymatic steps by E1 (ubiquitin activating), E2 (ubiquitin conjugating) and E3 (ubiquitin ligating) enzymes. E3 binds to substrate proteins and also to an E2 to form an E2-E3-substrate complex. It is the recognition and formation of said complex that has the highest level of substrate specificity for the conjugation cascade. The C-terminal Gly75-Gly76 residues of ubiquitin are the key residues that function in the diverse chemistry of ubiquitin reactions. Ubiquitin can be conjugated to itself via specific lysine (K6, K11, K27, K29, K33, K48 or K63) residues which result in diverse types of chain linkages.

Deubiquitination (Deconjugation)
The covalent ubiquitin bonds (isopeptide linkages) between ubiquitin and a target protein as well as between ubiquitin molecules in a chain can be reversed by specific deubiquitinating enzymes (DUBs). Recent studies have revealed that DUBs are dynamic enzymes that partner with various interacting proteins to facilitate both substrate selection and DUB activity. Assembly of individual DUBs into distinct protein complexes has allowed for the diversification of DUB activity that is needed to process the increasingly diverse assemblages of monoubiquitin and polyubiquitin marks on substrates. This dynamic regulation in the ubiquitin proteasome system is underscored by the increasing evidence that many DUBs are part of ubiquitin ligase complexes, which enables DUBs to regulate the activity and abundance of both the ligase and the substrate. A subset of DUBs and their associated complexes are displayed below, along with the cellular pathways in which they act.

Ubiquitin-Like Modifiers (UBLs)
Although ubiquitin is the most well understood post-translation modifier, there is a growing family of ubiquitin-like proteins (UBLs) that modify cellular targets in a pathway that is parallel to, but distinct from that of ubiquitin. These alternative modifiers include: SUMO, NEDD8, ISG15, APG8, APG12, FAT10, Ufm1, URM1, and Hub1. These related molecules have novel functions and influence diverse biological processes. There is also cross-regulation between the various conjugation pathways since some proteins can become modified by more than one UBL and sometimes even at the same lysine residue. For instance, SUMO modification often acts antagonistically to that of ubiquitination and serves to stabilize protein substrates. Proteins conjugated to UBLs are typically not targeted for degradation by the proteasome, but rather function in diverse regulatory activities. Attachment of UBLs might alter substrate conformation, affect the affinity for ligands or other interacting molecules, alter substrate localization and influence protein stability. UBLs are structurally similar to ubiquitin and are processed, activated, conjugated and released from conjugates by enzymatic steps that are similar to the corresponding mechanisms for ubiquitin. UBLs are also translated with C-terminal extensions that are processed to expose the invariant C-terminal LRGG. These modifiers have their own specific E1 (activating), E2 (conjugating) and E3 (ligating) enzymes that conjugate the UBLs to intracellular targets. These conjugates can be reversed by UBL-specific isopeptidases that have similar mechanisms to that of the deubiquitinating enzymes.
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Ordering Information

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**Mail:** R&D Systems, 614 McKinley Place NE, Minneapolis, MN 55413, USA

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Modification

R&D Systems reserves the right to make changes to these Terms and Conditions at any time without notice.
E1 Activating Enzymes

E1 proteins are a family of proteins called activating enzymes and are critical for the initiation of in vitro conjugation reactions for ubiquitin and ubiquitin-like proteins such as SUMO, NEDD8, ISG15, UFM1, FAT10 and Apg8 proteins. The conserved C-terminal glycine of ubiquitin is essential for activation by E1 and glycine residues are also found at the C-termini of other UBLs. This charged C-terminal residue eventually becomes conjugated to the lysyl ε-amino group of target proteins to form isopeptide linkages and subsequent conjugates.

The E1 for ubiquitin contains two active sites and activates the C-terminus of ubiquitin via a two-step, intra-molecular, ATP-dependent reaction. Initially, a tightly enzyme-bound ubiquitin adenylate (with PPi from ATP) is formed. This intermediate is then converted to form AMP and a covalent enzyme-ubiquitin thioester. Activation of a second ubiquitin molecule gives a complex with one equivalent each of ubiquitin thioester and tightly bound ubiquitin adenylate per subunit of enzyme. An alternative ubiquitin E1 (UBE1L2) exists and may control a distinct and differentially regulated ubiquitination pathway. The E1 activating enzymes for other UBLs function in an analogous manner.

Ubiquitin Activating Enzyme (UBE1), yeast

This E1 is responsible for the first step in ubiquitin-protein isopeptide bond formation, by forming a high-energy thioester bond with ubiquitin. The ubiquitin is activated by first adenylating with ATP its C-terminal glycine residue (Gly76) and thereafter linking this residue to the side chains of a cysteine residue in E1, yielding an ubiquitin-E1 thioester and free AMP. The activated ubiquitin is then transferred to a lysine of the targeted protein via the E2-E3 conjugation cascade. E1 is a critical component for the initiation of any in vitro conjugation reactions.

Purity: ≥95% (SDS-PAGE)  MW: ~110 kDa

His6-Ubiquitin Activating Enzyme (UBE1), human recombinant

This E1 is responsible for the first step in ubiquitin-protein isopeptide bond formation, by forming a high-energy thioester bond with ubiquitin. The ubiquitin is activated by first adenylating with ATP its C-terminal glycine residue (Gly76) and thereafter linking this residue to the side chains of a cysteine residue in UBE1, yielding an ubiquitin-UBE1 thioester and free AMP and PPi. The activated ubiquitin is then transferred to a lysine of the targeted protein via the E2-E3 conjugation cascade. UBE1 is a critical component for the initiation of any in vitro conjugation reactions. Protein has a His6 tag at the N-terminus.

Purity: ≥95% (SDS-PAGE)  MW: ~118 kDa

Ubiquitin Activating Enzyme (UBE1), human recombinant

This E1 is responsible for the first step in ubiquitin-protein isopeptide bond formation, by forming a high-energy thioester bond with ubiquitin. The ubiquitin is activated by first adenylating with ATP its C-terminal glycine residue (Gly76) and thereafter linking this residue to the side chains of a cysteine residue in E1, yielding an ubiquitin-E1 thioester and free AMP and PPi. The activated ubiquitin is then transferred to a lysine of the targeted protein via the E2-E3 conjugation cascade. E1 is a critical component for the initiation of any in vitro conjugation reactions.

Purity: ≥95% (SDS-PAGE)  MW: ~110 kDa
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Purity: ≥95% (SDS-PAGE)  MW: ~148 kDa

This E1 enzyme does not activate any other UBL proteins (including SUMO, NEDD8, ISG15, FAT10, FUB1, Uml1) and shows comparable rates of Ub activation to UBE1 in vitro. The enzyme specifically charges a newly identified E2 enzyme Ueb1 (Catalog # E2-677) which is not charged by UBE1. It can also charge other E2 enzymes including all UbcH5 isoforms and UbcH7. UBE1L2 is about 10-fold less abundant than UBE1 but likely represents an important alternate but distinct ubiquitin activating enzyme utilizes ATP to adenylate the C-terminal glycine residue of SUMO-1 (also SUMO-2 and SUMO-3), forming a high-energy thioester bond with the cysteine residue of Uba2 and the release of AMP and PPI. The second step is the trans-esterification reaction whereby SUMO-1 is transferred to Cys90 of UbcH9.

Purity: ≥90% (SDS-PAGE)  MW: ~140 kDa

The ATP-coupled activation of NEDD8 that is required for subsequent charging of the NEDD8-specific E2 UbcH12 is catalyzed by heterodimeric APPBP1-Uba3 in humans. The enzyme catalyzes the activation of the C-terminal carboxyl group of NEDD8 by forming a high-energy thioester bond in an ATP-dependent manner. Uba3 shows 43% homology to the C-terminal half of the ubiquitin activating E1 enzyme Uba1. The Uba3-catalyzed activation of NEDD8 exhibits an absolute requirement for APPBP1 which has high homology to the N-terminal half of Uba1.

Purity: ≥95% (SDS-PAGE)  MW: ~135 kDa

The ATP-coupled activation of NEDD8 that is required for subsequent charging of the NEDD8-specific E2 UbcH12 is catalyzed by heterodimeric APPBP1-Uba3 in humans. The enzyme catalyzes the activation of the C-terminal carboxyl group of NEDD8 by forming a high-energy thioester bond in an ATP-dependent manner. Uba3 shows 43% homology to the C-terminal half of the ubiquitin activating E1 enzyme Uba1. The Uba3-catalyzed activation of NEDD8 exhibits an absolute requirement for APPBP1 which has high homology to the N-terminal half of Uba1.

Purity: ≥95% (SDS-PAGE)  MW: ~109 kDa

Conjugation of the ubiquitin-like modifier SUMO (Sentrin) requires the activities of the heterodimeric E1 (Aos1/Uba2) and the UbcH9 E2 enzyme. The dimeric activating enzyme utilizes ATP to adenylate the C-terminal glycine residue of SUMO-1 (also SUMO-2 and SUMO-3), forming a high-energy thioester bond with the cysteine residue of Uba2 and the release of AMP and PPI. The second step is the trans-esterification reaction whereby SUMO-1 is transferred to Cys90 of UbcH9.

Purity: ≥90% (SDS-PAGE)  MW: ~110 kDa

For research use only. Not for use in diagnostic procedures.
**His$_6$-Apg E1 (Apg7L/ATG7), human recombinant**

E-317 25 µg

Autophagy is a process of bulk degradation of cytoplasmic components which are sequestered in double-membrane vesicles (autophagosomes) that deliver the contents to the lysosomal/vacuolar system for degradation. Apg7L is a homodimeric ubiquitin activating E1-like enzyme essential for Apg8 and Apg12 conjugation that mediates membrane fusion in autophagy. This enzyme activates the C-terminal glycine of both ubiquitin-like proteins (UBLs) Apg8 and Apg12 in an ATP-dependent manner. Its activity is essential for the conjugation of Apg8 proteins to the lipid phosphatidylethanolamine (PE), and the conjugation of Apg12L to Apg5L, and is a critical component for the initiation of any in vitro conjugation reactions.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~78.9 kDa

**His$_6$-UFM1 Activating Enzyme (UBA5), human recombinant**

E-319 25 µg

Uba5 is the E1-like enzyme responsible for the activation of the ubiquitin-like modifier, UFM1 (Catalog # UL-500). The enzyme contains a conserved ThiF domain with a nearby cysteine catalytic residue (Cys$^{250}$) and ATP-binding motif GXGXXG. This protein is highly conserved in humans and other higher eukaryotes, but not yeast. The enzyme utilizes ATP to adenylate the C-terminal glycine residue of UFM1, forming a high-energy thioester bond with the active site cysteine residue and the release of AMP. The second step is the trans-esterification reaction whereby UFM1 is transferred to the E2-like protein Ufc1 (Catalog # E2-675). This protein has an N-terminal His$_6$-tag.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~48 kDa

### E1 Activating Enzyme Inhibitors

**PYR 41**

2978 10 mg

A cell-permeable, irreversible ubiquitin-activating (E1) inhibitor that blocks ubiquitination and prevents ubiquitin-mediated proteasomal degradation. PYR 41 also inhibits NF-$\kappa$B activation, blocks degradation of p53, increases p21 levels and induces apoptosis in vitro. Also causes an increase in SUMOylation of proteins.

**Purity:** ≥98% (HPLC).  **FW:** ~371.3

### E1 Activating Enzyme Antibodies

#### ATG7

MAB6608 100 µg

**Species:** Human

**Description:** Monoclonal mouse IgG1, clone # 683906

**Application(s):** Western blot, Immunohistochemistry

#### UBE1L/ISG15 E1

A-306 100 µL

**Species:** Human

**Description:** Rabbit polyclonal

**Application(s):** Western blot, Immunohistochemistry

*For a full listing of antibodies, please see pages 89-90.*
E2 Conjugating Enzymes

The biochemical process of ubiquitin or ubiquitin-like (UBL) conjugation is initiated by the ATP-dependent E1 enzyme, followed by transfer to the active site cysteine of E2 enzymes (also called Ubc in yeast, or UbcH for human enzymes). These enzymes perform the second step in conjugation reactions by forming a thioester linkage with the C-terminal glycine. E2 enzymes function alone and in conjunction with E3 ligases to catalyze the attachment of ubiquitin to acceptor lysine residues of target proteins to form isopeptide bonds.

The identifying characteristic of an E2 is a 14-16 kDa core (UBC domain) that is about 35% conserved among family members. This portion of the enzyme contains an active site cysteine and substitution of this residue abolishes E2 activity. Enzymes consisting of the UBC domain only are class I E2s and those with C- or N-terminal extensions are class II and class III E2s, respectively. These extensions are thought to facilitate interactions with specific E3s. There are also E2 variants (UEVs) that lack a catalytic cysteine and function as heterodimers with E2s that contain functional active sites. Some E2 proteins function in pathways other than ubiquitination: UbcH9 functions with SUMO, UbcH12 with NEDD8, UbcH8 with ISG15, Apg3 with Apg8 and Ufc1 with UFM1. E2 proteins have various individual and redundant roles within the cell and have important roles in target degradation (proteasomal pathways) and target modification for regulatory purposes (non-proteasomal pathways).

Ubiquitination and Deubiquitination Cycle

UBE2K/E2-25K, human recombinant

E2-603

50 µg

100 µg

Biochemically, UbcH1 can catalyze the formation of K48-linked ubiquitin chains in the absence of an E3 ligase. This enzyme has a unique C-terminal UBA domain that plays a critical role in E1 selectivity and chain synthesis. Recently, UbcH1 (or HIP2) has been shown to bind to the N-terminus of huntingtin and may play a role in Huntington’s disease.

Purity: ≥95% (SDS-PAGE) MW: ~25 kDa

GST-UbcH2/UBE2H, human recombinant

E2-605

50 µg

100 µg

This E2 enzyme is the human homolog of the yeast Ubc8 gene. UbcH2 can conjugate ubiquitin to histone H2A in an E3-independent manner in vitro. This E2 enzyme is also involved in the ubiquitination of N-end rule pathway substrates, and can function in conjugation with the E3α ligase enzyme. Additionally, UbcH2 may have a role in sepsis-induced muscle protein proteolysis and cancer-induced cachexia. This protein has an N-terminal GST tag.

Purity: ≥95% (SDS-PAGE) MW: ~43 kDa

UbcH2/UBE2H, human recombinant

E2-607

50 µg

100 µg

This E2 enzyme is the human homolog of the yeast Ubc8 gene. UbcH2 can conjugate ubiquitin to histone H2A in an E3-independent manner in vitro. This E2 enzyme is also involved in the ubiquitination of N-end rule pathway substrates, and can function in conjugation with the E3α ligase enzyme. Additionally, UbcH2 may have a role in sepsis-induced muscle protein proteolysis and cancer-induced cachexia.

Purity: ≥95% (SDS-PAGE) MW: ~17 kDa

UbcH2/UBE2H, Dominant Negative, human recombinant

E2-608

100 µg

This E2 enzyme is the human homolog of the yeast Ubc8 gene. UbcH2 can conjugate ubiquitin to histone H2A in an E3-independent manner in vitro. This E2 enzyme is also involved in the ubiquitination of N-end rule pathway substrates, and can function in conjugation with the E3α ligase enzyme. Additionally, UbcH2 may have a role in sepsis-induced muscle protein proteolysis and cancer-induced cachexia.

Purity: ≥95% (SDS-PAGE) MW: ~17 kDa

Hisα-UbcH3/Cdc34, human recombinant

E2-610

50 µg

100 µg

UbcH3 plays an essential role in the progression of cells from the G1 to S phase of the cell division cycle. One pathway (requiring Cdc34) initiates DNA replication by degrading a CDK (cyclin-dependent kinase) inhibitor. The second pathway involves the anaphase-promoting complex (APC), initiates chromosome segregation and exit from mitosis by degrading anaphase inhibitors and mitotic cyclins.

Purity: ≥95% (SDS-PAGE) MW: ~27 kDa

Hisα-UbcH3/Cdc34, Dominant Negative, human recombinant

E2-611

100 µg

UbcH3 plays an essential role in the progression of cells from the G1 to S phase of the cell division cycle. One pathway (requiring Cdc34) initiates DNA replication by degrading a CDK (cyclin-dependent kinase) inhibitor. The second pathway involves the anaphase-promoting complex (APC), initiates chromosome segregation and exit from mitosis by degrading anaphase inhibitors and mitotic cyclins. The active site of this enzyme has been chemically inactivated for use as a negative or competitive control.

Purity: ≥95% (SDS-PAGE) MW: ~27 kDa
The human HR6A and HR6B proteins share about 95% amino acid sequence identity with each other and about 70% with yeast counterparts, but notably lack the acidic C-terminal domain found in *S. cerevisiae* proteins. The RAD6 pathway is essential to post-replication repair of DNA and damage-induced mutagenesis in eukaryotic cells. HR6 protein expression is cell cycle regulated and functions with RAD5 and RAD18 RING-Finger proteins. The human proteins function similarly to the yeast homologs, and may have additional roles in chromatin remodeling and spermatogenesis. Residue Ser120 is an important regulatory site in hHR6A, being phosphorylated *in vitro* by CDK1/2 on which increases ubiquitin conjugating activity. A nonsense mutation in hHR6A has been linked to a novel X-linked mental retardation syndrome (XMLR). This protein has an N-terminal His6-tag.

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~20 kDa

---

**Histidine Tagged-HR6B/UBE2B, human recombinant**

| E2-613 | 50 µg | 100 µg |

The human HR6A and HR6B proteins share about 95% amino acid sequence identity with each other and about 70% with yeast counterparts, but notably lack the acidic C-terminal domain found in *S. cerevisiae* proteins. The RAD6 pathway is essential to post-replication repair of DNA and damage-induced mutagenesis in eukaryotic cells. HR6 protein expression is cell cycle regulated and functions with RAD5 and RAD18 RING-Finger proteins. The human proteins function similarly to the yeast homologs, and may have additional roles in chromatin remodeling and spermatogenesis. Residue Ser120 is an important regulatory site in hHR6A, being phosphorylated *in vitro* by CDK1/2 on which increases ubiquitin conjugating activity. A nonsense mutation in hHR6A has been linked to a novel X-linked mental retardation syndrome (XMLR). This protein has an N-terminal His6-tag.

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~20 kDa

---

**GST-UbcH5a/UBE2D1, Dominant Negative, human recombinant**

| E2-617 | 100 µg |

Ubch5 enzymes are human homologs of the yeast UBC4/5 family and play many important regulatory roles in inflammation and cancer. Ubch5a mediates the degradation of a myriad of short-lived regulatory proteins (such as p53 in the presence of E6/E6-AP or MDM2, C-Fos, IκBα, p105) and abnormal proteins. Ubch5a has 89% and 88% sequence identity with Ubch5b and Ubch5c, respectively. This enzyme has a mutation of the active site from cysteine to serine which abolishes the ability of Ubch5a to transfer ubiquitin to an accepting E3 protein. Ideal for use as a negative or competitive control, or to study protein-protein interactions.

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~17 kDa

---

**Ubch5a/UBE2D1, Dominant Negative, human recombinant**

| E2-618 | 100 µg |

Ubch5 enzymes are human homologs of the yeast UBC4/5 family and play many important regulatory roles in inflammation and cancer. Ubch5a mediates the degradation of a myriad of short-lived regulatory proteins (such as p53 in the presence of E6/E6-AP or MDM2, C-Fos, IκBα, p105) and abnormal proteins. Ubch5a has 89% and 88% sequence identity with Ubch5b and Ubch5c, respectively. This enzyme has a mutation of the active site from cysteine to serine which abolishes the ability of Ubch5a to transfer ubiquitin to an accepting E3 protein. Ideal for use as a negative or competitive control, or to study protein-protein interactions.

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~43 kDa

---

**Ubch5b/UBE2D2, human recombinant**

| E2-620 | 50 µg | 100 µg |

Ubch5 enzymes are human homologs of the yeast UBC4/5 family and play many important regulatory roles in inflammation and cancer. Ubch5b mediates the degradation of a myriad of short-lived regulatory proteins (such as p53 in the presence of E6/E6-AP or MDM2, C-Fos, IκBα, p105) and abnormal proteins. Ubch5b has 89% and 88% sequence identity with Ubch5a and Ubch5c, respectively.

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~43 kDa

---

**Ubch5b/UBE2D2, human recombinant**

| E2-622 | 50 µg | 100 µg |

Ubch5 enzymes are human homologs of the yeast UBC4/5 family and play many important regulatory roles in inflammation and cancer. Ubch5b mediates the degradation of a myriad of short-lived regulatory proteins (such as p53 in the presence of E6/E6-AP or MDM2, C-Fos, IκBα, p105) and abnormal proteins. Ubch5b has 89% and 88% sequence identity with Ubch5a and Ubch5c, respectively.

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~17 kDa

---

**GST-UbcH5c/UBE2D3, human recombinant**

| E2-625 | 50 µg | 100 µg |

Ubch5 enzymes are human homologs of the yeast UBC4/5 family and play many important regulatory roles in inflammation and cancer. Ubch5c mediates the degradation of a myriad of short-lived regulatory proteins (such as p53 in the presence of E6/E6-AP or MDM2, C-Fos, IκBα, p105) and abnormal proteins. Ubch5c has 89% and 88% sequence identity with Ubch5a and Ubch5b, respectively.

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~43 kDa
UbcH5 enzymes are human homologs of the yeast UBC4/5 family and play many important regulatory roles in inflammation and cancer. UbcH5a mediates the degradation of a myriad of short-lived regulatory proteins (such as p53 in the presence of E6/E6-AP or MDM2, C-Fos, IκBα, p105) and abnormal proteins. UbcH5c has 88% and 89% sequence identity with UbcH5a and UbcH5b respectively.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~17 kDa

**His tag UbcH6/UBE2E1, human recombinant**

E2-630 50 µg  100 µg

UbcH6 mediates the selective degradation of short-lived and abnormal proteins. The enzyme has high similarity to UbcH5 E2 subfamily, but it does not participate in HECT-domain mediated events.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~21 kDa

**His tag UbcH6/UBE2E1, Dominant Negative, human recombinant**

E2-632 100 µg

UbcH6 mediates the selective degradation of short-lived and abnormal proteins. The enzyme has high similarity to UbcH5 E2 subfamily, but it does not participate in HECT-domain mediated events. This enzyme has a mutation of the active site from cysteine to serine which abolishes the ability of UbcH6 to transfer ubiquitin to an accepting E3 protein. Ideal for use as a negative or competitive control, or to study protein-protein interactions.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~21 kDa

**UbcH7/UBE2L3, human recombinant**

E2-640 50 µg  100 µg

UbcH7 mediates the selective degradation of short-lived and abnormal proteins and is highly homologous to UbcH5. UbcH7 interacts with the HECT domain of E6-AP and the RING domain of C-Cbl, and can mediate the multi-ubiquitination of many different types of protein substrates.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~18 kDa

**UbcH7/UBE2L3, Dominant Negative, human recombinant**

E2-642 100 µg

UbcH7 mediates the selective degradation of short-lived and abnormal proteins and is highly homologous to UbcH5. UbcH7 interacts with the HECT domain of E6-AP and the RING domain of C-Cbl, and can mediate the multi-ubiquitination of many different types of protein substrates. This enzyme has a mutation of the active site from cysteine to serine which abolishes the ability of UbcH7 to transfer ubiquitin to an accepting E3 protein. Ideal for use as a negative or competitive control, or to study protein-protein interactions.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~18 kDa

**GST-UbcH8/UBE2L6, human recombinant**

E2-643 50 µg  100 µg

UbcH8 can be charged with both ubiquitin and ISG15 via the activities of the respective E1β and E1α/β enzymes. UbcH8 is highly homologous to UbcH7, and functions in ubiquitin conjugation reactions and in HECT E3 (such as E6AP) and RING-FINGER (such as Parkin) protein mediated events. UbcH8 is the major E2 for ISG15 conjugation in reactions initiated by the ISG15-specific E1 activating enzyme. ISG15 is an ubiquitin-like protein that is conjugated to cellular proteins after IFN-α/β stimulation.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~44 kDa

**UbcH8/UBE2L6, human recombinant**

E2-644 50 µg  100 µg

UbcH8 can be charged with both ubiquitin and ISG15 via the activities of the respective E1β and E1α/β enzymes. UbcH8 is highly homologous to UbcH7, and functions in ubiquitin conjugation reactions and in HECT E3 (such as E6AP) and RING-FINGER (such as Parkin) protein mediated events. UbcH8 is the major E2 for ISG15 conjugation in reactions initiated by the ISG15-specific E1 activating enzyme. ISG15 is an ubiquitin-like protein that is conjugated to cellular proteins after IFN-α/β stimulation.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~18 kDa

**UBE21/Ubc9, human recombinant**

E2-645 50 µg  100 µg

UbcH9 is a member of the E2 family and is homologous to ubiquitin-conjugating enzymes, but is specific for the conjugation of SUMO to a variety of target proteins. This E2 is unusual in that it interacts directly with protein substrates that are modified by SUMOylation, and may play a role in substrate recognition. UbcH9 can mediate the conjugation of SUMO-1, SUMO-2 and SUMO-3 to a variety of proteins including RanGAP1, IκBα, and PML without the requirement of an E3 ligase.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~20 kDa

**UBE21/Ubc9, Dominant Negative, human recombinant**

E2-646 100 µg

UbcH9 is a member of the E2 family and is homologous to ubiquitin-conjugating enzymes, but is specific for the conjugation of SUMO to a variety of target proteins. This E2 is unusual in that it interacts directly with protein substrates that are modified by SUMOylation, and may play a role in substrate recognition. UbcH9 can mediate the conjugation of all SUMOs to a variety of proteins including RanGAP1, IκBα, and PML without the requirement of an E3 ligase. This enzyme has a mutation of the active site from cysteine to serine which abolishes the ability of UbcH9 to transfer ubiquitin to an accepting E3 protein. Ideal for use as a negative or competitive control, or to study protein-protein interactions.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~20 kDa

**His tag UbcH10/UBE2C, human recombinant**

E2-650 50 µg  100 µg

UbcH10 is an essential mediator of mitotic destruction events and cell cycle progression. It catalyzes the destruction of cyclins A and B in conjunction with the anaphase-promoting complex. This activity is essential at the end of mitosis for the inactivation of their partner kinase Cdc2 and exit from mitosis into G1 of the next cell cycle.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~20 kDa
<table>
<thead>
<tr>
<th><strong>His&lt;sub&gt;s&lt;/sub&gt;-UbcH10/UBE2C, Dominant Negative, human recombinant</strong></th>
<th><strong>His&lt;sub&gt;s&lt;/sub&gt;-UBE2N (Ubc13)/Uev1a Complex, human recombinant</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E2-652</strong></td>
<td><strong>E2-664</strong></td>
</tr>
<tr>
<td>100 µg</td>
<td>100 µg</td>
</tr>
</tbody>
</table>

UbcH10 is an essential mediator of mitotic destruction events and cell cycle progression. It catalyzes the destruction of cyclins A and B in conjunction with the anaphase-promoting complex. This activity is essential at the end of mitosis for the inactivation of their partner kinase Cdc2 and exit from mitosis into G1 of the next cell cycle. This enzyme has a mutation of the active site from cysteine to serine which abolishes the ability of UbcH10 to transfer ubiquitin to an accepting E3 protein. Ideal for use as a negative or competitive control, or to study protein-protein interactions.

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~17 kDa

<table>
<thead>
<tr>
<th><strong>GST-UBE2M/Ubc12, human recombinant</strong></th>
<th><strong>His&lt;sub&gt;s&lt;/sub&gt;-ApG3p/ATG3/Apg3L, human recombinant</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E2-655</strong></td>
<td><strong>E2-670</strong></td>
</tr>
<tr>
<td>50 µg</td>
<td>50 µg</td>
</tr>
</tbody>
</table>

GSTDylation requires the coordinated activities of UbcH12 and the APP-BP1/Uba3 activating enzyme. This E2 enzyme mediates the covalent conjugation of the ubiquitin-like protein NEDD8 to a limited number of cellular proteins such as Cdc53/Cul1 and other members of the cullin family (which are structural subunits of the Skp1-based and elongin B/C-based families of ubiquitin protein ligases). The NEDD8 pathway is essential for cell viability in fission yeast, and in mammalian cells it is essential for cell cycle progression and morphogenesis.

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~27 kDa

<table>
<thead>
<tr>
<th><strong>UBE2M/Ubc12, human recombinant</strong></th>
<th><strong>His&lt;sub&gt;s&lt;/sub&gt;-Ufc1, human recombinant</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E2-656</strong></td>
<td><strong>E2-675</strong></td>
</tr>
<tr>
<td>50 µg</td>
<td>50 µg</td>
</tr>
</tbody>
</table>

NEDDylation requires the coordinated activities of UbcH12 and the APP-BP1/Uba3 activating enzyme. This E2 enzyme mediates the covalent conjugation of the ubiquitin-like protein NEDD8 to a limited number of cellular proteins such as Cdc53/Cul1 and other members of the cullin family (which are structural subunits of the Skp1-based and elongin B/C-based families of ubiquitin protein ligases). The NEDD8 pathway is essential for cell viability in fission yeast, and in mammalian cells it is essential for cell cycle progression and morphogenesis.

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~20 kDa

<table>
<thead>
<tr>
<th><strong>His&lt;sub&gt;s&lt;/sub&gt;-UBE2N/Ubc13, human recombinant</strong></th>
<th><strong>His&lt;sub&gt;s&lt;/sub&gt;-Use1/UBE2Z, human recombinant</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E2-660</strong></td>
<td><strong>E2-677</strong></td>
</tr>
<tr>
<td>100 µg</td>
<td>50 µg</td>
</tr>
</tbody>
</table>

UbcH13 forms a heterodimeric complex with Uev1, and is able to assemble K63-linked poly-ubiquitin chains which have novel regulatory roles. These ubiquitin chains have a role in a variety of processes such as DNA repair, endocytosis, polysome stability and signal transduction pathways.

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~17 kDa

<table>
<thead>
<tr>
<th><strong>His&lt;sub&gt;s&lt;/sub&gt;-Uev1a (Mms2)/UBE2V1, human recombinant</strong></th>
<th><strong>His&lt;sub&gt;s&lt;/sub&gt;-UBE2E3, human recombinant</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E2-662</strong></td>
<td><strong>E2-678</strong></td>
</tr>
<tr>
<td>100 µg</td>
<td>50 µg</td>
</tr>
</tbody>
</table>

Ubiquitin E2 variant (UEV) proteins are similar to E2s in sequence but lack the active site cysteine residue. Uev1 is the human homolog of the yeast Mms2 protein. This particular UEV protein functions with the canonical E2 UbcH13 in the assembly of K63-linked poly-ubiquitin chains. These ubiquitin chains have a role in a variety of processes such as DNA repair, endocytosis, polysome stability and signal transduction pathways.

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~25 kDa

<table>
<thead>
<tr>
<th><strong>His&lt;sub&gt;s&lt;/sub&gt;-UbcH10/UBE2C, Dominant Negative, human recombinant</strong></th>
<th><strong>His&lt;sub&gt;s&lt;/sub&gt;-UBE2N (Ubc13)/Uev1a Complex, human recombinant</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E2-664</strong></td>
<td><strong>E2-664</strong></td>
</tr>
<tr>
<td>100 µg</td>
<td>100 µg</td>
</tr>
</tbody>
</table>

UbcH13 forms a heterodimeric complex with Uev1, and is able to assemble K63-linked poly-ubiquitin chains which have novel regulatory roles. These ubiquitin chains have a role in a variety of processes such as DNA repair, endocytosis, polysome stability and signal transduction pathways.

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~42 kDa

<table>
<thead>
<tr>
<th><strong>GST-UBE2M/Ubc12, human recombinant</strong></th>
<th><strong>His&lt;sub&gt;s&lt;/sub&gt;-ApG3p/ATG3/Apg3L, human recombinant</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E2-670</strong></td>
<td><strong>E2-670</strong></td>
</tr>
<tr>
<td>50 µg</td>
<td>50 µg</td>
</tr>
</tbody>
</table>


**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~36.8 kDa

<table>
<thead>
<tr>
<th><strong>His&lt;sub&gt;s&lt;/sub&gt;-Ufc1, human recombinant</strong></th>
<th><strong>His&lt;sub&gt;s&lt;/sub&gt;-Use1/UBE2Z, human recombinant</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E2-675</strong></td>
<td><strong>E2-677</strong></td>
</tr>
<tr>
<td>50 µg</td>
<td>50 µg</td>
</tr>
</tbody>
</table>

Ufc1 has been identified as the E2-like conjugating enzyme for the ubiquitin-like modifier, UFM1 (Catalog # UL-500). The protein was originally discovered in HEK293 cells by immunoprecipitation using Flag-Uba5 (Catalog # E-319), the E1-like activating enzyme of UFM1. Ufc1 shares no homology to other E2 enzymes except for the region around the active site cysteine (Cys<sup>114</sup>) which is capable of forming a thioester bond with UFM1. This protein is N-terminally tagged.

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~22.6 kDa

<table>
<thead>
<tr>
<th><strong>His&lt;sub&gt;s&lt;/sub&gt;-UbcH10/UBE2C, Dominant Negative, human recombinant</strong></th>
<th><strong>His&lt;sub&gt;s&lt;/sub&gt;-UBE2E3, human recombinant</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E2-678</strong></td>
<td><strong>E2-678</strong></td>
</tr>
<tr>
<td>50 µg</td>
<td>50 µg</td>
</tr>
</tbody>
</table>

UbcE3 is a class III E2 protein with unique C- and N-terminal extension in addition to the conserved UBC domain. This enzyme shares over 90% identity with UbcH6, mouse UbcM2 and Drosophila UbcD2, but the protein N-termini are divergent. This E2 enzyme is active in conjugation reactions with various E3 ligase enzymes (BRCA1, CHIP), and is known to interact with two RING-FINGER proteins ARA54 and RNF8 via its UBC domain. This protein is N-terminally tagged.

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~20.5 kDa

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**E2 Conjugating Enzymes**

### His<sub>6</sub>-UBE2F, human recombinant

E2-679 50 µg 100 µg

UBE2F accepts the ubiquitin-like protein NEDD8 from the NEDD8 E1 complex and catalyzes its covalent attachment to other proteins. The specific interaction with the E3 ubiquitin ligase RBX2, but not RBX1, suggests that the RBX2-UBE2F complex modifies specific target proteins, such as CUL5. UbcH12 (UBE2M) is another NEDD8 E2 conjugating enzyme which interacts with RBX1 and targets different types of cullin substrates. The UBE2F and UBE2M enzymes thus represent a hierarchical expansion of the NEDD8 conjugation system in establishing selective culling RING ligase activation which influences substrate sub-type NEDDylation. This protein is N-terminally tagged.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~20.5 kDa

### His<sub>6</sub>-UBE2G2, human recombinant

E2-680 50 µg 100 µg

Ube2G2 is a Class I E2 enzyme and shares 60% and 100% sequence identity with S.cerevisiae Ubc7 and mouse respectively. In yeast, Ubc7 is a endoplasmic reticulum (ER)-bound molecule and the active site faces the cytosol. The Ube2G2 E2 enzyme and the GP78 E3 ligase are active components of ERAD (endoplasmic reticulum-associated degradation) pathway which is essential for the degradation of misfolded ER proteins. The mechanism of K48-linked poly-ubiquitination by Ube2G2/GP78 appears to involve the transfer of preassembled Ub chains from Ube2G2 to lysine residues in a substrate. The E2 and E3 enzymes form a large hetero-oligomer which brings multiple Ube2G2 molecules into close proximity which allows for Ub transfer between neighboring E2s. This protein is N-terminally tagged.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~20.5 kDa

### His<sub>6</sub>-UBE2S, human recombinant

E2-690 50 µg 100 µg

UBE2S (E2-24K, E2-EPF) shares 38% identity with S.cerevisiae Ubc4 with a conserved UBC domain characteristic of ubiquitin E2 conjugating enzymes. This E2 enzyme is over-expressed in common human cancers, suggesting an involvement in oncogenesis, and targets von Hippel-Lindau (VHL) for proteosomal degradation in cells, thereby stabilizing hypoxia-inducible factor (HIF)-1α for ubiquitin-mediated destruction. Ube2S levels correlate inversely with pVHL level in most tumor cell lines. Overexpression of Ube2S in vitro and in vivo boosts tumor-cell proliferation, invasion and metastasis through effects on the pVHL-HIF pathway, and may thus be a novel molecular target for therapeutic intervention. This protein is N-terminally tagged.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~20.5 kDa

### His<sub>6</sub>-UBE2T, human recombinant

E2-695 50 µg 100 µg

UBE2T (HSPC150) was cloned from CD34-positive hematopoietic stem cells. UBE2T homologs are only found in vertebrates and have widespread expression. UBE2T is the E2 enzyme which is essential for the Fanconi anemia pathway, required for the efficient repair of damaged DNA. UBE2T binds to FANCL, the ubiquitin ligase subunit of the Fanconi anemia core complex, and is required for the mono-ubiquitination of FANCD2 in vivo. In addition, UBE2T can mono-ubiquitinate itself in vivo which inactivates the E2, UBE2T. This self-inactivation mechanism could be important for negative regulation of the Fanconi anemia pathway. This protein is N-terminally tagged.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~20.5 kDa

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**E2 Conjugating Enzyme Antibodies**

### ATG3

AF5450 100 µg

**Species:** Human/Mouse/Rat  **Form:** Antigen affinity purified sheep polyclonal  **Application(s):** Western blot

### ATG10

AF5464 100 µg

**Species:** Human  **Form:** Antigen affinity purified sheep polyclonal  **Application(s):** Western blot

### UbcH1/UBE2K/E2-25K

A-603 200 µL

**Species:** Human  **Description:** Protein A affinity purified rabbit polyclonal  **Application(s):** Western blot

### UbcH2/UBE2H

A-605 200 µL

**Species:** Human  **Description:** Protein A affinity purified rabbit polyclonal  **Application(s):** Western blot

### UbcH3/Cdc34

A-610 200 µL

**Species:** Human  **Description:** Protein A affinity purified rabbit polyclonal  **Application(s):** Western blot

### UbcH5/UBE2D

A-615 200 µL

**Species:** Human  **Description:** Protein A affinity purified rabbit polyclonal  **Application(s):** Western blot

### UbcH6/UBE2E1

A-630 200 µL

**Species:** Human  **Description:** Protein A affinity purified rabbit polyclonal  **Application(s):** Western blot

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For research use only. Not for use in diagnostic procedures.
UbcH7/UBE2L3

A-640 200 µL
Species: Human
Form: Protein A affinity purified rabbit polyclonal
Application(s): Western blot

UbcH9/UBE2E3

A-645 200 µL
Species: Human
Form: Protein A affinity purified rabbit polyclonal
Application(s): Western blot

UbcH10/UBE2C

A-650 200 µL
Species: Human
Form: Affinity purified rabbit polyclonal
Application(s): Western blot

UbcH12/UBE2M

A-655 200 µL
Species: Human
Form: Protein A affinity purified rabbit polyclonal
Application(s): Western blot

UBE2K/E2-25K

AF6609 100 µg
Species: Human/Mouse/Rat
Form: Antigen affinity purified sheep polyclonal
Application(s): Western blot

MAB6609 100 µg
Species: Human/Mouse
Form: Monoclonal mouse IgG2b, Clone # 701316
Application(s): Western blot, Immunohistochemistry

For a full listing of antibodies, please see pages 89-90.
**E3 Ligase Enzymes**

Ubiquitin modification of substrate proteins is achieved by the activity of E1 activating, E2 conjugating and E3 ligase enzymes. Substrate proteins can be modified with one molecule (mono-ubiquitination) or many molecules (poly-ubiquitination) of ubiquitin and each type of modification has a distinct regulatory fate. In addition, many E3 mediated pathways have been implicated in human disease and are attractive targets for drug discovery. However, currently no small molecule modulator candidates for this class of enzymes have reached the clinic. Each E3 enzyme targets a small number of proteins for Ub modification, but the exact substrates are mostly unknown and their identification continues to be a challenge.

E3 ligase enzymes are a large (> 500) and complex super-family, many of which contain multiple binding domains to interact with ubiquitin, E2 enzymes and substrate proteins. In addition to substrate ubiquitination, many E3 ligases can also self- or auto-ubiquitinate in the presence of an E2 enzyme, a property that may be used as an auto-regulatory mechanism to control their own intracellular levels. In general, the detailed molecular mechanism, stoichiometries and linkage site selection of E3 enzymes are poorly understood. As with ubiquitin E3 ligases, similar activities are also part of the final conjugation processes for other UBL proteins. There are E3 enzymes that are specific for targets that are modified SUMO, NEDD8, ISG15 and presumably also for FAT10 and UFM1.

Ubiquitin E3 enzymes are classified into two primary classes according to domain homology and mechanism of action. The subtypes include the HECT (homologous to E6-AP C-terminus) and RING (Really Interesting New Gene) proteins. The HECT E3 ligases contain a large domain (~ 350 residues) with a catalytic cysteine residue that transfers Ub via a cognate E2 directly to the substrate. These enzymes interact directly with substrate target proteins to effect poly-ubiquitination. Examples of HECT proteins include the viral E6AP, ARB-BP1, Itch, NEDD4, Smurfl2 and WWPI. The RING E3 ligases have two zinc ions coordinated by multiple Cysteine and Histidine residues to form a globular E2-binding domain. In contrast to HECT E3s, RING E3s do not have the recognizable catalytic active sites that define “classical” enzymes. Instead, these E3s have large binding interfaces and act as scaffold proteins that bring together the participant E2 and substrate proteins. Examples of RING-FINGER proteins include BRCA1, Cbl, Efp, Hdm2, MurF1, Parkin, SIAH, TRAF6, Rfn11 and XIAP. Two other RING-FINGER related domains, the U-box (UFD2-homology domain) and PHD (plant homeo domain) also possess E3 activity. U-box E3s (CHIP, UFD2, PRP19, UIP5 and CYC4) have a similar RING-FINGER tertiary structure and may participate in protein quality control via their interaction with chaperones. PHD containing proteins (C-MIR, AIRE, MR1 and MR2) have E3 activity that is PHD domain-dependent, but it is not known if all PHD proteins function similarly. Another group of E3s are multi-component complexes, such as the modular SCF (Skp1/Cullin/F-box/Rbx1/2) family. These complexes are exemplified by the Rbx RING-FINGER proteins in various combinations with at least three components including a Cullin protein, an adaptor (Sk1, Elongin B/C or a BTB) and a substrate binding protein (Fbox, SOCS or BTB). The APC (anaphase-promoting complex) is also a large multiprotein-E3 complex that regulates both entry and exit from mitosis via the ubiquitination of key cell cycle regulators, such as cyclin B and securin. This large complex (~ 11 subunits) is similar to SCF and contains a catalytic core cullin (Apc2) and a RING protein (Apc11).

**E3 Ligase Enzyme Inhibitors**

<table>
<thead>
<tr>
<th>E3 Ligase Enzyme Inhibitors</th>
<th>E3-100</th>
<th>25 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>MurF1 (Muscle-specific RING-FINGER protein 1) is a RING-FINGER E3 ligase found in striated muscle (heart and skeletal) and iris tissues. The N-terminal E3 ligase activity regulates the proteasomal degradation of cardiac troponin and probably other sarcomeric-associated proteins. MuRF1 may have a role in muscle adaptation, atrophy and hypertrophy mediated by UPP pathways. Muscle atrophy is associated with many diseases including cancer, diabetes and AIDS. This protein is N-terminally tagged.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purity: ≥95% (SDS-PAGE)</td>
<td>MW: ~40 kDa</td>
<td></td>
</tr>
</tbody>
</table>

**His<sup>6</sup>-Parkin, human recombinant**

<table>
<thead>
<tr>
<th>E3-150</th>
<th>50 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutations in the Parkin (PRKN2) gene is considered to be a major cause of autosomal recessive juvenile parkinsonism (AR-PJ). Parkin is an E3 ligase with a N-terminal ubiquitin-like motif and a C-terminal RING domain composed of two RING-FINGER motifs separated by two IBR domains. Parkin can auto-ubiquitinate itself and ubiquitinate various substrates in an E2-dependent manner, targeting them for degradation. Parkin functions with E2 enzymes UbcH7 (Catalog # E2-640), UbcH8 (Catalog # E2-644) and UbcH13/Uev1 (Catalog # E2-664). Parkin disease-associated mutations often affect E3 ligase activity which may thus result in the neurotoxic accumulation of misfolded proteins. This protein is N-terminally tagged.</td>
<td></td>
</tr>
<tr>
<td>Purity: ≥95% (SDS-PAGE)</td>
<td>MW: ~52 kDa</td>
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</table>

**His<sup>6</sup>-MuRF1/TRIM63, human recombinant**

<table>
<thead>
<tr>
<th>E3-100</th>
<th>25 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>MurF1 (Muscle-specific RING-FINGER protein 1) is a RING-FINGER E3 ligase found in striated muscle (heart and skeletal) and iris tissues. The N-terminal E3 ligase activity regulates the proteasomal degradation of cardiac troponin and probably other sarcomeric-associated proteins. MuRF1 may have a role in muscle adaptation, atrophy and hypertrophy mediated by UPP pathways. Muscle atrophy is associated with many diseases including cancer, diabetes and AIDS. This protein is N-terminally tagged.</td>
<td></td>
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<tr>
<td>Purity: ≥95% (SDS-PAGE)</td>
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**E3 Ligase Enzyme Inhibitors**

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>3503</td>
<td>10 mg</td>
<td>50 mg</td>
</tr>
<tr>
<td>Inhibitor of Hdm2 ubiquitin ligase (E3). Blocks Hdm2-mediated ubiquitylation and proteasomal degradation of p53; activates p53-dependent transcription. Induces apoptosis in several tumor cell lines that express wild-type p53, such as LOX-MVI, A549, HT1080 and U2OS.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purity: ≥99% (HPLC).</td>
<td>MW: ~377.87</td>
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</table>

<table>
<thead>
<tr>
<th>NSC 66811</th>
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<tbody>
<tr>
<td>2936</td>
<td>10 mg</td>
<td>50 mg</td>
</tr>
<tr>
<td>Purity: ≥97% (HPLC).</td>
<td>MW: ~340.42</td>
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### NSC 146109 Hydrochloride

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<th></th>
<th>10 mg</th>
<th>50 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cell-permeable, genotype-selective anti-tumor agent that activates p53-dependent transcription. Increases levels of endogenous p53 in tumor cells and protects p53 from MDM2-mediated degradation. Displays some selectivity for tumor cells vs. normal cells in an MTT cell viability assay.

**Purity:** ≥99% (HPLC). **MW:** ~316.85

### ProTAME

<table>
<thead>
<tr>
<th></th>
<th>1 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cell-permeable prodrug capable of inducing mitotic arrest and cell death in HeLa and other cell types. proTAME is converted to its active parent molecule (TAME, tosyl-L-arginine methyl ester) by intracellular esterases. TAME inhibits the ubiquitin ligase activity of the anaphase-promoting complex/cyclosome (APC/C) by preventing its activation by Cdc20 and Cdh1.

**Purity:** ≥90% (HPLC). **MW:** ~726.75

### SMER 3

<table>
<thead>
<tr>
<th></th>
<th>10 mg</th>
<th>50 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Specific inhibitor of a yeast SCF family E3 ubiquitin ligase (SCF<sup>Met30</sup>) in vitro and in vivo. Induces the expression of MET genes; blocks cell proliferation. Enhancer of Rapamycin.

**Purity:** ≥98% (HPLC).

### Thalidomide

<table>
<thead>
<tr>
<th></th>
<th>100 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Teratogen, sedative-hypnotic with inherent anti-inflammatory properties. A thalidomide-binding protein, cereblon (CRBN), forms an E3 ubiquitin ligase complex with damaged DNA binding protein 1 (DDB1) and Cul4A that is important for limb outgrowth and expression of the fibroblast growth factor FGF-8 in zebrafish and chicks. Thalidomide initiates its teratogenic effects by binding to CRBN and inhibiting the associated ubiquitin ligase activity.

**Purity:** ≥99% (HPLC). **MW:** ~258.23

### E3 Ligase Enzyme Antibodies

#### BRCA1 C-Terminus

- **AF6955** 100 µL
- **Species:** Human
- **Form:** Antigen affinity purified sheep polyclonal
- **Application(s):** Immunohistochemistry

#### BRCA1 N-Terminus

- **AF2210** 100 µg
- **Species:** Human
- **Form:** Antigen affinity purified goat polyclonal
- **Application(s):** Western blot, Immunohistochemistry

#### Phospho-BRCA1 (S1423)

- **AF1386** 100 µg
- **Species:** Human
- **Form:** Antigen affinity purified rabbit polyclonal
- **Application(s):** Western blot, Immunohistochemistry

#### CBL

- **AF5998** 100 µg
- **Species:** Human/Mouse
- **Form:** Antigen affinity purified goat polyclonal
- **Application(s):** Western blot

#### GRAIL/RNF128

- **AF6234** 100 µg
- **Species:** Human
- **Form:** Antigen affinity purified sheep polyclonal
- **Application(s):** Western blot

#### MARCH8

- **MAB8696** 100 µg
- **Species:** Human/Mouse/Rat
- **Form:** Monoclonal mouse IgG<sub>2a</sub>, Clone # 710424
- **Application(s):** Western blot, Immunocytochemistry
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<tr>
<th><strong>E3 LIGASE ENZYMES</strong></th>
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</thead>
</table>

<table>
<thead>
<tr>
<th><strong>MDM2/HDM2</strong></th>
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</thead>
<tbody>
<tr>
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<tr>
<td><strong>Species:</strong> Human/Mouse/Rat</td>
</tr>
<tr>
<td><strong>Form:</strong> Antigen affinity purified rabbit polyclonal</td>
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<tr>
<td><strong>Application(s):</strong> Western blot, Immunohistochemistry</td>
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<tr>
<td>BAF1244 50 µg</td>
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<tr>
<td><strong>Species:</strong> Human/Mouse/Rat</td>
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<tr>
<td><strong>Form:</strong> Biotinylated antigen affinity purified rabbit polyclonal</td>
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<tr>
<td><strong>Application(s):</strong> Western blot</td>
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<table>
<thead>
<tr>
<th><strong>Parkin</strong></th>
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<tbody>
<tr>
<td>AF1438 100 µg</td>
</tr>
<tr>
<td><strong>Species:</strong> Human</td>
</tr>
<tr>
<td><strong>Form:</strong> Antigen affinity purified goat polyclonal</td>
</tr>
<tr>
<td><strong>Application(s):</strong> Western blot, Immunohistochemistry</td>
</tr>
<tr>
<td>MAB1438 100 µg</td>
</tr>
<tr>
<td><strong>Species:</strong> Human</td>
</tr>
<tr>
<td><strong>Form:</strong> Monoclonal mouse IgG2B, Clone # 323302</td>
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<tr>
<td><strong>Application(s):</strong> Western blot</td>
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<tr>
<td>MAB14381 100 µg</td>
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<tr>
<td><strong>Species:</strong> Human</td>
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<tr>
<td><strong>Form:</strong> Monoclonal mouse IgG2A, Clone # 328122</td>
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<td><strong>Application(s):</strong> Western blot</td>
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<table>
<thead>
<tr>
<th><strong>Mind Bomb 2/MIB2</strong></th>
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<tbody>
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<tr>
<td><strong>Form:</strong> Monoclonal mouse IgG2B, Clone # 197702</td>
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<tr>
<td><strong>Application(s):</strong> Immunoprecipitation</td>
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</table>

<table>
<thead>
<tr>
<th><strong>MuRF1/TRIM63</strong></th>
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</thead>
<tbody>
<tr>
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<td><strong>Species:</strong> Human/Mouse/Rat</td>
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<tr>
<td><strong>Form:</strong> Antigen affinity purified goat polyclonal</td>
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<tr>
<td><strong>Application(s):</strong> Western blot</td>
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<table>
<thead>
<tr>
<th><strong>PIAS2</strong></th>
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</thead>
<tbody>
<tr>
<td>AF4768 100 µg</td>
</tr>
<tr>
<td><strong>Species:</strong> Human</td>
</tr>
<tr>
<td><strong>Form:</strong> Antigen affinity purified sheep polyclonal</td>
</tr>
<tr>
<td><strong>Application(s):</strong> Western blot</td>
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<table>
<thead>
<tr>
<th><strong>PIAS3</strong></th>
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</thead>
<tbody>
<tr>
<td>AF5120 100 µg</td>
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<td><strong>Species:</strong> Human</td>
</tr>
<tr>
<td><strong>Form:</strong> Antigen affinity purified goat polyclonal</td>
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<tr>
<td><strong>Application(s):</strong> Western blot</td>
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<table>
<thead>
<tr>
<th><strong>NEDD4</strong></th>
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</thead>
<tbody>
<tr>
<td>AF6218 100 µg</td>
</tr>
<tr>
<td><strong>Species:</strong> Human/Mouse/Rat</td>
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<tr>
<td><strong>Form:</strong> Antigen affinity purified sheep polyclonal</td>
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<tr>
<td><strong>Application(s):</strong> Western blot</td>
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<tr>
<td>MAB6218 100 µg</td>
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<tr>
<td><strong>Species:</strong> Human/Mouse/Rat</td>
</tr>
<tr>
<td><strong>Form:</strong> Monoclonal mouse IgG2B, Clone # 683211</td>
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<tr>
<td><strong>Application(s):</strong> Western blot</td>
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<table>
<thead>
<tr>
<th><strong>RN2F</strong></th>
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<tbody>
<tr>
<td>AF6065 100 µg</td>
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<tr>
<td><strong>Species:</strong> Human/Mouse/Rat</td>
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<tr>
<td><strong>Form:</strong> Antigen affinity purified goat polyclonal</td>
</tr>
<tr>
<td><strong>Application(s):</strong> Western blot</td>
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<table>
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<tr>
<th><strong>RN168</strong></th>
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<tbody>
<tr>
<td>AF7217 100 µL</td>
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<tr>
<td><strong>Species:</strong> Human/Mouse</td>
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<tr>
<td><strong>Form:</strong> Antigen affinity purified sheep polyclonal</td>
</tr>
<tr>
<td><strong>Application(s):</strong> Western blot, Immunohistochemistry</td>
</tr>
</tbody>
</table>
**Skp2**

AF6817 100 µL  
Species: Human  
Form: Antigen affinity purified sheep polyclonal  
Application(s): Western blot

**SMURF2**

MAB6916 100 µg  
Species: Human/Mouse/Rat  
Form: Monoclonal mouse IgG2a, Clone # 690638  
Application(s): Western blot

**STUB1/CHIP**

AF4685 100 µg  
Species: Human  
Form: Antigen affinity purified goat polyclonal  
Application(s): Western blot

**TRIM21**

AF6219 100 µg  
Species: Human  
Form: Antigen affinity purified sheep polyclonal  
Application(s): Western blot, Immunohistochemistry

**TRIM32**

AF6515 100 µL  
Species: Human/Mouse  
Form: Antigen affinity purified sheep polyclonal  
Application(s): Western blot

**UBR5**

AF6404 100 µg  
Species: Human/Mouse/Rat  
Form: Antigen affinity purified sheep polyclonal  
Application(s): Western blot

For a full listing of antibodies, please see pages 89-90.

---

**E3 Ligase Enzyme ELISA Kits**

**Total MDM2**

<table>
<thead>
<tr>
<th>Kit Code</th>
<th>Plates</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
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<td>2 Plates</td>
</tr>
<tr>
<td>DYC1244-5</td>
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</tr>
<tr>
<td>DYC1244E</td>
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Species: Human
Deconjugating Enzymes

Protein ubiquitination is a reversible process and the deconjugation reactions are performed by specific cysteine proteases which generate monomeric ubiquitin from a variety of C-terminal adducts. Deubiquitinating enzymes (DUBs) are the largest family of enzymes in the ubiquitin system with diverse functions, making them key regulators of ubiquitin-mediated pathways and they often function by direct or indirect association with the proteasome. The activity of DUBs has been implicated in several important pathways, including cell growth and differentiation, development, oncogenesis, neuronal disease and transcriptional regulation.

DUBs catalyze the removal of ubiquitin from native conjugates, ubiquitin C-terminal extension peptides and linear poly-ubiquitin fusion or precursor proteins. Many DUBs have esterase and amidase activities in vitro, and ubiquitin-esters or ubiquitin-amidocoumarins (ubiquitin-AMCs) have been widely used as general substrates for activity assays. These activities could be physiologically relevant for the removal of linkages that might arise in vivo from nucleophilic attack by small molecules (e.g., glutathione and spermidine) on reactive ubiquitin thioester linkages.

DUBs are classed into two distinct families: ubiquitin C-terminal hydrolases (UCHs) and the ubiquitin-specific proteases (USPs/UBPs). UCHs are relatively small enzymes (20-30 kDa) that catalyze the removal of peptides and small molecules from the C-terminus of ubiquitin. Most UCHs cannot generate monomeric ubiquitin from protein conjugates or disassemble poly-ubiquitin chains. USPs vary greatly in size (50-300 kDa), usually with N-terminal extensions which may function in substrate recognition, subcellular localization and protein-protein interactions. USPs can process ubiquitin precursors, remove ubiquitin from protein conjugates and disassemble ubiquitin chains. Isopeptidase T (IsoT) is a USP capable of binding ubiquitin and specifically disassembles free or unanchored poly-ubiquitin chains. Most recently the JAMM isopeptidases, otubains and ataxin-3/josephin have also been identified as ubiquitin-specific proteases. There are also enzymes that specifically hydrolyze C-terminal isopeptide bonds for UBLs: SUMO-specific proteases (SEPNPs), NEDD8-specific proteases (COP9 signalosome), ISG15-specific proteases (UBP43), UFM1-specific proteases (UfsP1, UfsP2) and Apg-specific proteases.

**Isopeptidase T/USP5, rabbit**

| E-320 | 10 µg | 25 µg |

Isopeptidase T is a member of the high molecular weight USP family that catalyzes the hydrolysis of amides, esters and thioesters from the C-terminus of ubiquitin. This enzyme primarily disassembles free poly-ubiquitin chains linked through isopeptide bonds. This activity has a critical role in the recycling of Ub and in the regulation of the activity of the 26S proteasome.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~97 kDa

**Isopeptidase T/USP5, human recombinant**

| E-322 | 25 µg |

Isopeptidase T is a member of the high molecular weight USP family that catalyzes the hydrolysis of amides, esters and thioesters from the C-terminus of ubiquitin. This enzyme primarily disassembles free poly-ubiquitin chains linked through isopeptide bonds. This activity has a critical role in the recycling of Ub and in the regulation of the activity of the 26S proteasome.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~97 kDa

**Isopeptidase T/USP5, short form, human recombinant**

| E-324 | 25 µg |

Isopeptidase T is a member of the high molecular weight USP family that catalyzes the hydrolysis of amides, esters and thioesters from the C-terminus of ubiquitin. This enzyme primarily disassembles free poly-ubiquitin chains linked through isopeptide bonds. This activity has a critical role in the recycling of Ub and in the regulation of the activity of the 26S proteasome. This isoform results from a splicing variant of the long isoform, and differs by a substitution of 23 amino acids (G629-S652) with a single alanine.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~97 kDa
UCH-L3, human recombinant

E-325  25 µg

Ubiquitin C-terminal hydrolases are a family of cysteine hydrolases that catalyze the hydrolysis of amides, esters and thioesters from the C-terminus of ubiquitin. UCH-L3 is a member of the low molecular weight UCHs that processes ubiquitin precursors and ubiquitinated proteins to generate monomeric ubiquitin. This enzyme is also able to cleave at the C-terminus of NEDD8.

Purity: ≥95% (SDS-PAGE)  MW: ~26 kDa

UCH-L3, rabbit

E-330 10 µg
E-330 25 µg

UCH-L3 is a member of the low molecular weight UCHs that processes ubiquitin precursors and ubiquitinated proteins to generate monomeric ubiquitin. This enzyme is also able to cleave at the C-terminus of NEDD8.

Purity: ≥95% (SDS-PAGE)  MW: ~30 kDa

His6-UCH-L1, mouse recombinant

E-335 25 µg

UCH-L1 is a member of the low molecular weight UCHs that processes ubiquitin precursors and ubiquitinated proteins to generate monomeric ubiquitin. The expression of UCH-L1 is highly specific to cells of the neuroendocrine system and their tumors. UCH-L1 variants (Ile93Met) have been linked to Parkinson's disease and may participate in processes critical for neuronal health.

Purity: ≥95% (SDS-PAGE)  MW: ~25 kDa

His6-Ataxin-3, human recombinant

E-341 50 µg

Defects in the ATXN3 gene are the cause of Machado-Joseph disease (MJD), also known as spinocerebellar ataxia 3. MJD is an autosomal dominant neurodegenerative disorder caused by an expansion of a (CAG) repeat in the ATXN3 gene resulting in polymorphic protein forms in the C-terminal polyglutamine region. Ataxin-3 protein belongs to a novel group of cysteine proteases similar to USP-type ubiquitin proteases and has deubiquitinating activity in vitro. The full-length protein contains an N-terminal Josephin domain, two ubiquitin interacting motifs (UIMs), and a variable C-terminus consisting of a polyglutamine stretch and tail. Ataxin-3 activity may have a role in MJD pathogenesis by influencing ubiquitin-dependent pathways that control protein folding and stability. This protein is N-terminally tagged.

Purity: ≥95% (SDS-PAGE)  MW: ~42 kDa

Disassembly of free ubiquitin chains by deconjugating enzymes. Enzymes (1 µM) were incubated with 4 µg of ubiquitin chains at 37 °C.
**His<sub>6</sub>-A20/TNFAIP3 Catalytic Domain, human recombinant**

<table>
<thead>
<tr>
<th>MW (µg)</th>
<th>Purity</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>E-344</td>
<td>≥95% (SDS-PAGE)</td>
<td>~46 kDa</td>
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</table>

A20 (TNFα-induced protein 3) is a cytoplasmic zinc finger protein that inhibits NF-κB activity and tumor necrosis factor-mediated programmed cell death. The protein interacts with NAF1 and inhibits TNFα-induced NF-κB-dependent gene expression by interfering with receptor-interacting protein (RIP) - or TRAF2-mediated transactivation signalling. A20 contains an N-terminal domain which has deubiquitinating enzyme activity and removes ubiquitin chains from RIP, thus mediating distinct regulatory effects in the down-regulation of NFκB-signalling representing the catalytic domain (CD) of A20. This protein is N-terminally tagged and contains residues 1-371 of A20.

**His<sub>6</sub>-BAP1/RNF2, human recombinant**

<table>
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<th>MW (µg)</th>
<th>Purity</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-345</td>
<td>≥95% (SDS-PAGE)</td>
<td>~81 kDa</td>
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</tbody>
</table>

BAP1 (BRCA1 Associated Protein 1) interacts with the ring-finger domain of the E3 ligase BRCA1 (Breast Cancer 1, early onset protein), which functions as a tumor suppressor in the BRCA1 growth control pathway. The N-terminal 240 amino acids of the predicted 729-αmino acid human protein show homology to ubiquitin C-terminal hydrolases (UCHs) and recombinant BAP1 has deubiquitinating activity in vitro. In addition, BAP1 contains an acidic region, a highly charged C-terminal region, and 2 putative nuclear localization signals. It has been demonstrated that BAP1 and BRCA1 associate in vivo and have overlapping sub-nuclear localization patterns. BAP1 appears to be a key regulator of the BRCA1 growth control pathway and has been proposed to be a novel candidate tumor suppressor. This protein is N-terminally tagged.

**His<sub>6</sub>-A20 Catalytic Domain, human recombinant**

<table>
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<tr>
<td>E-506</td>
<td>≥95% (SDS-PAGE)</td>
<td>~42 kDa</td>
</tr>
</tbody>
</table>

USP2 (Ubiquitin Specific Protease 2) is a cysteine protease over-expressed in prostate cancer, is androgen-regulated and interacts with and prolongs the half-life of fatty acid synthase (FAS). FAS stabilization is associated with the malignancy of a subset of aggressive prostate cancers. MDM2 is also a substrate for USP2 indicating that the enzyme may regulate p53-dependent pathways. The in vivo and in vitro oncogenic properties of USP2 are also linked to its deubiquitinating activity. It has been hypothesized that USP2 might stabilize short-lived proteins that accumulate and may act as regulators or effectors in the induction of apoptosis. This recombinant protein is a C-terminally tagged and contains residues 259-605 representing the catalytic domain (CD) of USP2.

**His<sub>6</sub>-BAP1/RNF2 Catalytic Domain, human recombinant**

<table>
<thead>
<tr>
<th>MW (µg)</th>
<th>Purity</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-518</td>
<td>≥95% (SDS-PAGE)</td>
<td>~42 kDa</td>
</tr>
</tbody>
</table>

USP7 (Herpes-Associated Ubiquitin Specific Protease) is a nuclear protein that was initially identified as a novel p53-interacting protein. The enzyme deubiquitinates p53 thus stabilizing the levels of this key tumor suppressor, and inducing p53-dependent cell growth repression and apoptosis. USP7 also indirectly affects p53 stability by deubiquitinating HDMX, a regulator of HDM2. Full-length USP7 (1102 amino acids) contains an N-terminal p53 binding domain and 2 highly conserved USP active site domains. The C-terminal domain interacts with HSV-1 protein ICP0. This enzyme is able to cleave model substrates including linear Ub fusions, poly-ubiquitinated EBNA1 and poly-ubiquitinated p53. This recombinant protein is N-terminally tagged and contains residues 213-548 representing the catalytic domain (CD) of USP7.

**His<sub>6</sub>-A20 Catalytic Domain, human recombinant**

<table>
<thead>
<tr>
<th>MW (µg)</th>
<th>Purity</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-519</td>
<td>≥95% (SDS-PAGE)</td>
<td>~128 kDa</td>
</tr>
</tbody>
</table>

USP7 (Herpes-Associated Ubiquitin Specific Protease) is a nuclear protein that was initially identified as a novel p53-interacting protein. The enzyme deubiquitinates p53 thus stabilizing the levels of this key tumor suppressor, and inducing p53-dependent cell growth repression and apoptosis. USP7 also indirectly affects p53 stability by deubiquitinating HDMX, a regulator of HDM2. Full-length USP7 (1102 amino acids) contains an N-terminal p53 binding domain and 2 highly conserved USP active site domains. The C-terminal domain interacts with HSV-1 protein ICP0. This enzyme is able to cleave model substrates including linear Ub fusions, poly-ubiquitinated EBNA1 and poly-ubiquitinated p53. This recombinant protein is N-terminally tagged.

**His<sub>6</sub>-BAP1/RNF2 Catalytic Domain, human recombinant**

<table>
<thead>
<tr>
<th>MW (µg)</th>
<th>Purity</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-520</td>
<td>≥95% (SDS-PAGE)</td>
<td>~131 kDa</td>
</tr>
</tbody>
</table>

USP8 (Ubiquitin Specific Protease 8) is a growth-regulated deubiquitinating enzyme with a role in endosomal sorting of receptor tyrosine kinases (RTKs). The C-terminus contains the DUB catalytic center and a nearby Zn⁺ ribbon subdomain which might have a role in poly-Ub binding specificity and substrate-induced conformational changes. USP8 has DUB activity on K48- and K63-linked Ub chains in vitro. This protein is N-terminally tagged.
**His<sub>6</sub>-Otubain-1, human recombinant**

| E-622 | 50 μg |

Otubains belong to the ovarian tumor (OTU) protein super-family present in eukaryotes, viruses and pathogenic bacterium. These proteins have no obvious homology to DUBs, however Otubain1 and Otubain2 were identified by affinity purification with the DUB-specific inhibitor Ub-aldehyde and both have an OTU domain (containing the active site, UIMs and UBAs), Ub-interacting motifs (UIMs) and Ub-associated domains (UBAs). Otubain1 exists in two isoforms with differential functions. This protein is N-terminally tagged. Accession # NP_060140.

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~33.2 kDa

**USP14, human recombinant**

| E-544 | 25 μg |

USP14, the human homolog of the yeast Ubp6 protein, is a member of the family of thiol proteases involved in the hydrolysis of ubiquitin C-terminal protein derivatives. USP14 has a low-affinity association with the proteasome (PA700, 19S cap particle), and this association increases its basal enzyme activity. The physiological role of USP14 may be the release of ubiquitin from proteasome-bound conjugates. 

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~56 kDa

**His<sub>6</sub>-UBP43/USP18, human recombinant**

| E-600 | 50 μg |

UBP43 has homology to the catalytic domains of ubiquitin-specific proteases (USPs) and is the only deconjugating enzyme identified to date that cleaves ISG15 from substrates. The enzyme acts upon ISG15 fusions including native ISG15 conjugates linked via isopeptide bonds. This enzyme is induced by type I interferon (IFN). This protein is N-terminally tagged.

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~44 kDa

**GST-AMSH**

| E-550 | 25 μg |

Coming soon.

**His<sub>6</sub>-SENP1 Catalytic Domain, human recombinant**

| E-700 | 50 μg |

The covalent modification of proteins by SUMO is reversible and is mediated by SENP enzymes. SENPs process SUMO precursor proteins to generate the mature and active form. These enzymes also remove poly-SUMO conjugates from substrate proteins. SENP1 is active against SUMO-1, SUMO-2 and SUMO-3 in vitro, but not against ubiquitin or NEDD8. This protein is N-terminally tagged and contains residues 415-643 representing the catalytic domain of SENP1.

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~29.8 kDa

**His<sub>6</sub>-SENP2 Catalytic Domain, human recombinant**

| E-710 | 50 μg |

The covalent modification of proteins by SUMO is reversible and is mediated by SENP enzymes. SENPs process SUMO precursor proteins to generate the mature and active form. These enzymes also remove poly-SUMO conjugates from substrate proteins. SENP2 is active against SUMO-1, SUMO-2 and SUMO-3 in vitro but not against ubiquitin or NEDD8. This protein is N-terminally tagged and contains residues 368-593 representing the catalytic domain of SENP2.

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~28.7 kDa

**His<sub>6</sub>-NEDP1/SENP8, human recombinant**

| E-800 | 50 μg |

The covalent modification of proteins by NEDD8 is reversible and is mediated by NEDP1. NEDP1 cleaves residues from the C-terminus of NEDD8 precursor proteins to generate the mature and active form which contains the conserved C-terminal di-glycine. This enzyme also reverses the process of NEDDylation by removing poly-NEDD8 conjugates from substrate proteins. NEDP1 has been shown to deconjugate NEDD8 from CUL2 in vitro and from CUL4A in vivo, and does not show activity against ubiquitin or SUMO proteins. This protein is N-terminally tagged.

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~29.5 kDa

---

**Table: Deconjugating Enzymes**

<table>
<thead>
<tr>
<th>SUMO Type</th>
<th>SENP Chain Type</th>
<th>SENP Chain Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>SENP1&lt;sub&gt;CD&lt;/sub&gt;</td>
<td>SUMO (3-8) mixture</td>
<td>SENP2&lt;sub&gt;CD&lt;/sub&gt;</td>
</tr>
<tr>
<td>SUMO Monomer</td>
<td>SUMO-2</td>
<td>SUMO-3</td>
</tr>
</tbody>
</table>

Deconjugation of SUMO-2 and SUMO-3 chains by SUMO-specific isopeptidases SENP1 and SENP2. Reactions contained 2 μg of SUMO chains in 50 mM Hepes, 50 mM NaCl, 5 mM DTT and were incubated in the presence of 500 nM SENP1 (S1) or SENP2 (S2) for 60 minutes at 37˚C.
### Deconjugating Enzyme Substrates

**Ubiquitin AMC, human recombinant**

U-550 50 µg

Fluorogenic substrate for ubiquitin hydrolases based on the C-terminal derivatization of ubiquitin with 7-amido-4-methylcoumarin (AMC). Ubiquitin AMC is an exquisitely sensitive substrate for UCH-L3 (K<sub>m</sub> = 0.039 µM) and for Isopeptidase-T (K<sub>m</sub> = 0.17 - 1.4 µM). Ub-AMC is useful for studying ubiquitin hydrolyses when detection sensitivity or continuous monitoring of activity is essential.

**Purity:** ≥95% (HPLC)  **MW:** ~8.6 kDa

**Ubiquitin AFC, human recombinant**

U-551 50 µg

Fluorogenic substrate based on the C-terminus derivatization of ubiquitin with 7-amino-4-trifluoromethylcoumarin (AFC). Similar to ubiquitin AMC, this is an exquisitely sensitive deubiquitinating enzyme substrate and is useful for studying ubiquitin C-terminal hydrolytic activity when detection sensitivity or continuous monitoring is essential. The fluorophore has a larger Stokes radius than AMC which is useful to reduce compound interference in HTS assays.

**Purity:** ≥95% (HPLC)  **MW:** ~8.6 kDa

**Ubiquitin-Rhodamine110 (R110), human recombinant**

U-555 50 µg

Fluorogenic substrate for deubiquitinating enzymes based on the C-terminal derivatization of ubiquitin with Rhodamine110 (R110). Similar to other C-terminus derivatives such as Ub-AMC (Catalog # U-550) and Ub-AFC (Catalog # U-551), this is an exquisitely sensitive deubiquitinating enzyme substrate and is useful for studying ubiquitin C-terminal hydrolytic activity when detection sensitivity or continuous monitoring of activity at longer wavelengths is essential.

**Purity:** ≥95% (HPLC)  **MW:** ~8.5 kDa

**Ubiquitin Aminoluciferin (AML), human recombinant**

U-556 50 µg

Substrate for deubiquitinating enzymes based on the C-terminal derivative of ubiquitin with aminoluciferin (AML). Rather than fluorescence as the indicator of DUB activity, DUB liberated luciferin is processed by luciferase to give a luminescent signal. Ub-AML not only produces a stronger signal, but also has an excellent signal to noise ratio over traditional fluorophores. This makes it possible to rapidly assess the activity of DUBs that poorly utilize Ub-AMC while using much lower levels of the DUBs themselves.

**Purity:** ≥95% (SDS PAGE);  **MW:** ~8.8 kDa

**Ubiquitin-Lys-TAMRA**

**(Ub-Gly76c-Lys-TAMRA-Gly-OH)**

U-558 50 µg

Fluorescence polarization high-throughput screening (HTS) reagent which is based on a 5-tetramethylrhodamine (TAMRA) modified Lys-Gly sequence that is linked to ubiquitin via a native isopeptide bond with the lysine side-chain. This reagent is useful for studying ubiquitin C-terminal hydrolytic activity when detection sensitivity or continuous monitoring of activity at longer wavelengths is essential.

**Purity:** ≥95% (HPLC)  **MW:** ~9.2 kDa

**Di-Ub (K11-linked) FRET TAMRA Pos4, human recombinant**

UF-440 50 µg

Linkage specific diubiquitin is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. These FRET-based DUB substrates with isopeptide bonds between ubiquitin moieties are superior to gel densitometry assays. Since DUBs recognize and cleave substrates with specific steric conditions, each substrate varies in the position of the fluorophore and quencher and must be empirically tested for individual enzymes. Ubiquitination through K11 is important for endoplasmic reticulum-associated degradation (ERAD) and there undoubtedly exist DUBs that may have specificity for K11 linkages.

**Purity:** ≥95% (HPLC);  **MW:** ~17 kDa

**Di-Ub (K48-linked) FRET TAMRA Pos1, human recombinant**

UF-210 50 µg

Linkage specific diubiquitin is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. These FRET-based DUB substrates with isopeptide bonds between ubiquitin moieties are superior to gel densitometry assays. Since DUBs recognize and cleave substrates with specific steric conditions, each substrate varies in the position of the fluorophore and quencher and must be empirically tested for individual enzymes.

**Purity:** ≥95% (HPLC);  **MW:** ~17 kDa

**Di-Ub (K48-linked) FRET EDANS Pos1, human recombinant**

UF-211 50 µg

Linkage specific diubiquitin is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. These FRET-based DUB substrates with isopeptide bonds between ubiquitin moieties are superior to gel densitometry assays. Since DUBs recognize and cleave substrates with specific steric conditions, each substrate varies in the position of the fluorophore and quencher and must be empirically tested for individual enzymes.

**Purity:** ≥95% (HPLC);  **MW:** ~17 kDa

**Di-Ub (K48-linked) FRET TAMRA Pos2, human recombinant**

UF-220 50 µg

Linkage specific diubiquitin is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. These FRET-based DUB substrates with isopeptide bonds between ubiquitin moieties are superior to gel densitometry assays. Since DUBs recognize and cleave substrates with specific steric conditions, each substrate varies in the position of the fluorophore and quencher and must be empirically tested for individual enzymes.

**Purity:** ≥95% (HPLC);  **MW:** ~17 kDa
Di-Ub (K48-linked) FRET EDANS Pos2, 
*human recombinant*

**UF-221**  
50 µg

Linkage specific diubiquitin is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. These FRET-based DUB substrates with isopeptide bonds between ubiquitin moieties are superior to gel densitometry assays. Since DUBs recognize and cleave substrates with specific steric conditions, each substrate varies in the position of the fluorophore and quencher and must be empirically tested for individual enzymes.

**Purity:** ≥95% (HPLC)  
**MW:** ~17 kDa

---

Di-Ub (K48-linked) FRET TAMRA Pos3, 
*human recombinant*

**UF-230**  
50 µg

Linkage specific diubiquitin is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. These FRET-based DUB substrates with isopeptide bonds between ubiquitin moieties are superior to gel densitometry assays. Since DUBs recognize and cleave substrates with specific steric conditions, each substrate varies in the position of the fluorophore and quencher and must be empirically tested for individual enzymes.

**Purity:** ≥95% (HPLC)  
**MW:** ~17 kDa

---

Di-Ub (K48-linked) FRET EDANS Pos3, 
*human recombinant*

**UF-231**  
50 µg

Linkage specific diubiquitin is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. These FRET-based DUB substrates with isopeptide bonds between ubiquitin moieties are superior to gel densitometry assays. Since DUBs recognize and cleave substrates with specific steric conditions, each substrate varies in the position of the fluorophore and quencher and must be empirically tested for individual enzymes.

**Purity:** ≥95% (HPLC)  
**MW:** ~17 kDa

---

Di-Ub (K63-linked) FRET TAMRA Pos1, 
*human recombinant*

**UF-310**  
50 µg

Linkage specific diubiquitin is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. These FRET-based DUB substrates with isopeptide bonds between ubiquitin moieties are superior to gel densitometry assays. Since DUBs recognize and cleave substrates with specific steric conditions, each substrate varies in the position of the fluorophore and quencher and must be empirically tested for individual enzymes.

**Purity:** ≥95% (HPLC)  
**MW:** ~17 kDa

---

Di-Ub (K63-linked) FRET EDANS Pos1, 
*human recombinant*

**UF-311**  
50 µg

Linkage specific diubiquitin is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. These FRET-based DUB substrates with isopeptide bonds between ubiquitin moieties are superior to gel densitometry assays. Since DUBs recognize and cleave substrates with specific steric conditions, each substrate varies in the position of the fluorophore and quencher and must be empirically tested for individual enzymes.

**Purity:** ≥95% (HPLC)  
**MW:** ~17 kDa

---

Di-Ub (K63-linked) FRET TAMRA Pos2, 
*human recombinant*

**UF-320**  
50 µg

Linkage specific diubiquitin is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. These FRET-based DUB substrates with isopeptide bonds between ubiquitin moieties are superior to gel densitometry assays. Since DUBs recognize and cleave substrates with specific steric conditions, each substrate varies in the position of the fluorophore and quencher and must be empirically tested for individual enzymes.

**Purity:** ≥95% (HPLC)  
**MW:** ~17 kDa

---

Di-Ub (K63-linked) FRET TAMRA Pos3, 
*human recombinant*

**UF-330**  
50 µg

Linkage specific diubiquitin is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. These FRET-based DUB substrates with isopeptide bonds between ubiquitin moieties are superior to gel densitometry assays. Since DUBs recognize and cleave substrates with specific steric conditions, each substrate varies in the position of the fluorophore and quencher and must be empirically tested for individual enzymes.

**Purity:** ≥95% (HPLC)  
**MW:** ~17 kDa

---

HA-GABARAP/Apg8p1 AMC, *human recombinant*

**UL-440**  
25 µg

Fluorogenic substrate for Apg8 specific C-terminal hydrolases such as Apg4b (Catalog # E-400) based on the C-terminal derivatization of Apg8 with 7-amido-4-methylcoumarin (AMC). Apg8-AMC is useful for studying such enzyme activities when detection sensitivity or continuous monitoring of activity is essential. This protein has an N-terminal HA-tag.

**Purity:** ≥90% (HPLC)  
**MW:** ~14 kDa

---

HA-GATE-16/Apg8p2 AMC, *human recombinant*

**UL-445**  
25 µg

Fluorogenic substrate for Apg8 specific C-terminal hydrolases such as Apg4b (Catalog # E-400) based on the C-terminal derivatization of Apg8 with 7-amido-4-methylcoumarin (AMC). Apg8-AMC is useful for studying such enzyme activities when detection sensitivity or continuous monitoring of activity is essential. This protein has an N-terminal HA-tag.

**Purity:** ≥90% (HPLC)  
**MW:** ~14 kDa

---

HA-LC3/MAP1LC3A/Apg8p3 AMC, *human recombinant*

**UL-450**  
25 µg

Fluorogenic substrate for Apg8 specific C-terminal hydrolases such as Apg4b (Catalog # E-400) based on the C-terminal derivatization of Apg8 with 7-amido-4-methylcoumarin (AMC). Apg8-AMC is useful for studying such enzyme activities when detection sensitivity or continuous monitoring of activity is essential. This protein has an N-terminal HA-tag.

**Purity:** ≥90% (HPLC)  
**MW:** ~14.4 kDa
**SUMO-1 AMC, human recombinant**

UL-551 50 µg

Fluorogenic substrate for SUMO-1 hydrolases based on the C-terminus derivatization of SUMO-1 with 7-amido-4-methylcoumarin (AMC). SUMO-1 AMC is useful for studying SUMO-1 hydrolases when detection sensitivity or continuous monitoring of activity is essential.

**Purity:** ≥95% (HPLC) **MW:** ~11.6 kDa

---

**SUMO-1 Aminoluciferin (AML), human recombinant**

UL-704 50 µg

Substrate for SUMO deconjugating enzymes (SENP) based on the C-terminal derivative of SUMO with aminoluciferin (AML). Rather than fluorescence as the indicator of SENP activity, SENP liberated luciferin is processed by luciferase to give a luminescence signal. SUMO-AML not only produces a stronger signal, but also has an excellent signal to noise ratio over traditional fluorophores. This makes it possible to rapidly assess the activity of SENPs that poorly utilize SUMO-AML while using much lower levels of the SENPs themselves.

**Purity:** ≥95% (HPLC) **MW:** ~11.3 kDa

---

**SUMO-2 AMC, human recombinant**

UL-758 50 µg

Fluorogenic substrate for SUMO-2 hydrolases based on the carboxy-terminus derivatization of SUMO-2 with 7-amido-4-methylcoumarin (AMC). SUMO-2 AMC is useful for studying SUMO-2 hydrolases (SENP) when detection sensitivity or continuous monitoring of activity is essential. NOTE: This protein contains an N-terminal HA-tag.

**Purity:** ≥95% (HPLC) **MW:** ~11 kDa

---

**SUMO-3 AMC, human recombinant**

UL-768 50 µg

Fluorogenic substrate for SUMO-3 hydrolases based on the carboxy-terminus derivatization of SUMO-3 with 7-amido-4-methylcoumarin (AMC). SUMO-3 AMC is useful for studying SUMO-3 hydrolases (SENP) when detection sensitivity or continuous monitoring of activity is essential. This protein contains an N-terminal HA-tag.

**Purity:** ≥95% (HPLC) **MW:** ~11 kDa

---

**NEDD8 AMC, human recombinant**

UL-552 50 µg

Fluorogenic substrate for NEDD8 hydrolases based on the carboxy-terminus derivatization of NEDD8 with 7-amido-4-methylcoumarin (AMC). NEDD8 AMC is useful for studying NEDD8 hydrolases when detection sensitivity or continuous monitoring of activity is essential.

**Purity:** ≥95% (HPLC) **MW:** ~9 kDa

---

**NEDD8 Aminoluciferin (AML), human recombinant**

UL-804 50 µg

Substrate for deconjugating enzymes (NEDPs) based on the C-terminal derivative of NEDD8 with aminoluciferin (AML). Rather than fluorescence as the indicator of NEDP activity, NEDP liberated luciferin is processed by luciferase to give a luminescence signal. NEDD8-AML not only produces a stronger signal, but also has an excellent signal to noise ratio over traditional fluorophores. This makes it possible to rapidly assess the activity of NEDPs that poorly utilize NEDD8-AML while using much lower levels of the NEDPs themselves.

**Purity:** ≥95% (HPLC) **MW:** ~8.8 kDa

---

**ISG15 AMC, human recombinant**

UL-553 50 µg

Fluorogenic substrate for ISG15 hydrolases based on the carboxy-terminus derivatization of ISG15 with 7-amido-4-methylcoumarin (AMC). ISG15 AMC is useful for studying enzymes (such as UBP43) when detection sensitivity or continuous monitoring of activity is essential.

**Purity:** ≥95% (HPLC) **MW:** ~17 kDa

---

**Z-Leu-Arg-Gly-Gly-AMC, human recombinant**

S-100 5 mg

Fluorogenic tetra-peptide substrate for ubiquitin C-terminal hydrolases (UCHs, e.g. UCHL3) and Isopeptidase T. This low molecular weight peptide represents a minimal sequence of C-terminal residues of ubiquitin where hydrolysis occurs at the UbGly76-X bond. The catalytic efficiency for hydrolysis of this substrate by deconjugating enzymes is several thousand-fold less than a more native substrate (Ub-AMC, Catalog # U-550) since remote interactions with Ub-containing substrates stabilize catalytic transition states.

**Purity:** ≥95% (HPLC) **MW:** ~692.76

---

**Deconjugating Enzyme Inhibitors**

**Ubiquitin Aldehyde, human recombinant**

U-201 50 µg

A potent and highly specific inhibitor of all ubiquitin C-terminal hydrolases. This protein blocks the hydrolysis of poly-ubiquitin chains on substrate proteins in vitro and thus enhances poly-ubiquitin chain accumulation. Inhibits UCH Isopeptidase T (K<sub>i</sub>=2.5 nM).

**Purity:** ≥96% (HPLC) **MW:** ~8.5 kDa

---

**Ubiquitin Vinyl Sulfone, human recombinant**

U-202 50 µg

A potent, irreversible and specific inhibitor of all ubiquitin C-terminal hydrolases (UCHs), ubiquitin-specific proteases (USPs) and deubiquitinating enzymes (DUBs). Useful for inhibiting the hydrolysis of poly-ubiquitin chains on substrate proteins in vitro and thus enhances poly-ubiquitin chain accumulation.

**Purity:** ≥98% (HPLC) **MW:** ~8.6 kDa

---

**Ubiquitin Vinyl Methyl Ester, human recombinant**

U-203 50 µg

A potent, irreversible and specific inhibitor of all ubiquitin C-terminal hydrolases (UCHs), ubiquitin-specific proteases (USPs) and deubiquitinating enzymes (DUBs). Useful for inhibiting the hydrolysis of poly-ubiquitin chains on substrate proteins in vitro and thus enhances poly-ubiquitin chain accumulation.

**Purity:** ≥98% (HPLC) **MW:** ~8.6 kDa

---

*For research use only. Not for use in diagnostic procedures.*
### HA-Ubiquitin Aldehyde, human recombinant

**U-211**

50 µg

Ubiquitin-aldehyde is a potent and specific inhibitor of most deubiquitinating enzymes (DUBs) such as ubiquitin C-terminal hydrolases (UCHs) and ubiquitin-specific proteases (USPs). It prevents the hydrolysis of poly-ubiquitin chains on substrate proteins in vitro and thus enhances poly-ubiquitin chain accumulation. This tagged version contains an N-terminal HA peptide sequence (YPYDVPDYA) derived from the influenza hemagglutinin protein. This epitope allows for the sensitive identification or purification of DUBs since it is specifically recognized by anti-HA antibodies and/or anti-HA-agarose.

**Purity:** ≥95% (HPLC) **MW:** ~9.5 kDa

### HA-Ubiquitin Vinyl Sulfone, human recombinant

**U-212**

25 µg

This N-terminal HA-tagged ubiquitin is a potent, irreversible and specific inhibitor of most deubiquitinating enzymes (DUBs) including ubiquitin C-terminal hydrolases (UCHs) and ubiquitin-specific proteases (USPs). Useful for inhibiting the hydrolysis of poly-ubiquitin chains on substrate proteins in vitro and thus enhances poly-ubiquitin chain accumulation. The HA peptide sequence (YPYDVPDYA) is derived from the influenza hemagglutinin protein. This epitope allows for the sensitive identification or purification of DUBs since it is specifically recognized by anti-HA antibodies and/or anti-HA-agarose.

**Purity:** ≥95% (HPLC) **MW:** ~9.5 kDa

### SUMO-1 Aldehyde, human recombinant

**UL-701**

50 µg

A potent and highly specific inhibitor of SUMO-specific isopeptidases (SENPs). This protein blocks the hydrolysis of poly-SUMO chains on substrate proteins in vitro and thus enhances poly-SUMO chain accumulation.

**Purity:** ≥98% (HPLC) **MW:** ~11.1 kDa

### HA-SUMO-2 Vinyl Sulfone, human recombinant

**UL-759**

50 µg

This N-terminal HA-tagged SUMO is a potent, irreversible and specific inhibitor of SUMO-specific isopeptidases (SENPs). This protein inhibits the hydrolysis of poly-SUMO chains on substrate proteins in vitro and thus enhances poly-SUMO chain accumulation. The HA peptide sequence (YPYDVPDYA) is derived from the influenza hemagglutinin protein. This epitope allows for the sensitive identification or purification of SENP activities since it is specifically recognized by anti-HA antibodies and/or anti-HA-agarose.

**Purity:** ≥90% (HPLC) **MW:** ~10.6 kDa

### NSC 632839 Hydrochloride

**2647**

10 mg

50 mg

Inhibitor of ubiquitin isopeptidase activity that displays no effect on the proteolytic activity of the proteasome. Induces apoptosis via a Bcl-2-dependent and apoptosis-independent pathway of caspase activation.

**Purity:** ≥99% (HPLC) **MW:** ~316.05 kDa

### IU1 Small Molecule USP14 Inhibitor (SMI-USP14),

**I-300**

10 mg

50 mg

USP14 is a DUB enzyme which can inhibit the degradation of ubiquitin-protein conjugates both in vitro and in cells. This compound is a cell-permeable USP14 inhibitor which leads to increased proteasome activity. Enhancement of proteasome activity through inhibition of USP14 may offer a strategy to reduce the levels of aberrant proteins in cells under proteotoxic stress.

**Purity:** ≥95% (HPLC) **MW:** ~300.4 kDa

### HA-GABARAP/Apg8p1 Vinyl Sulfone, human recombinant

**UL-441**

25 µg

This N-terminal HA-tagged Apg8 protein is a potent, irreversible and specific inhibitor of Agp8-specific isopeptidases (such as Agp84B, Catalog # E-400). Agp84B activities include the processing of Agp8 precursor proteins and the removal of Agp8 conjugates. These processes can be inhibited by this VS derivative which reacts with the Agp84B active site cysteine. The HA peptide sequence (YPYDVPDYA) is derived from the influenza hemagglutinin protein. This epitope allows for identification and purification using anti-HA antibodies and/or anti-HA-agarose.

**Purity:** ≥90% (HPLC) **MW:** ~14 kDa
For research use only. Not for use in diagnostic procedures.

### HA-GATE-6/Apg8p2 Vinyl Sulfone, human recombinant

**UL-446** 25 µg

This N-terminal HA-tagged Apg8 protein is a potent, irreversible and specific inhibitor of Apg8-specific isopeptidases (such as Apg84B, Catalog # E-400). Apg84B activities include the processing of Apg8 precursor proteins and the removal of Apg8 conjugates. These processes can be inhibited by this VS derivative which reacts with the Apg84B active site cysteine. The HA peptide sequence (YPYDVPDYA) is derived from the influenza hemagglutinin protein. This epitope allows for identification and purification using anti-HA antibodies and/or anti-HA-agarose.

**Purity:** ≥90% (HPLC)  **MW:** ~13.8 kDa

### HA-LC3/MAP1LC3A/Apg8p3 Vinyl Sulfone, human recombinant

**UL-451** 25 µg

This N-terminal HA-tagged Apg8 protein is a potent, irreversible and specific inhibitor of Apg8-specific isopeptidases (such as Apg84B, Catalog # E-400). Apg84B activities include the processing of Apg8 precursor proteins and the removal of Apg8 conjugates. These processes can be inhibited by this vinyl sulfone derivative which reacts with the Apg84B active site cysteine. The HA peptide sequence (YPYDVPDYA) is derived from the influenza hemagglutinin protein. This epitope allows for identification and purification using anti-HA antibodies and/or anti-HA-agarose.

**Purity:** ≥90% (HPLC)  **MW:** ~14.4 kDa

### ISG15 Aldehyde, human recombinant

**UL-602** 50 µg

A potent and highly specific inhibitor of ISG15-specific isopeptidases (such as UBP43). This protein blocks the hydrolysis of poly-ISG15 chains on substrate proteins in vitro and thus enhances poly-ISG15 chain accumulation.

**Purity:** ≥95% (HPLC)  **MW:** ~17 kDa

### ISG15 Vinyl Sulfone, human recombinant

**UL-603** 50 µg

A potent and highly specific inhibitor of ISG15-specific isopeptidases (such as UBP43). This protein blocks the hydrolysis of poly-ISG15 chains on substrate proteins in vitro and thus enhances poly-ISG15 chain accumulation.

**Purity:** ≥95% (HPLC)  **MW:** ~17 kDa

### HA-ISG15 Vinyl Sulfone, human recombinant

**UL-604** 50 µg

This N-terminal HA-tagged ISG15 is a potent, irreversible and specific inhibitor of ISG15-specific isopeptidases (such as UBP43). This protein blocks the hydrolysis of poly-ISG15 chains on substrate proteins in vitro and thus enhances poly-ISG15 chain accumulation. The HA peptide sequence (YPYDVPDYA) is derived from the influenza hemagglutinin protein. This epitope allows for the sensitive identification or purification of such deconjugating activities since it is specifically recognized by anti-HA antibodies and/or anti-HA-agarose.

**Purity:** ≥90% (HPLC)  **MW:** ~17 kDa

### Summary of Deubiquitinating Enzyme Activity

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Ub-AMC kcat/km (M⁻¹ sec⁻¹)</th>
<th>K48-linked Ub chains</th>
<th>K63-linked Ub chains</th>
<th>Ub-H/vS Inhibition</th>
<th>NEDD8-H/vS Inhibition</th>
<th>SUMO-H/vS Inhibition</th>
<th>ISG15-H/vS Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bap1</td>
<td>2.4 x 10⁶</td>
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<tr>
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</tbody>
</table>

Assays containing 5 nM enzyme (except for USP1/UAF1 [1 nM each] and UCHL3 [10 µM]) were incubated with Ub-AMC at 25 °C in 50 mM Heps pH 8, 100 mM NaCl, 5 mM DTT, 1% glycerol, 0.1 mg/mL BSA. Fluorescent signals were converted to product formation using a free AMC standard curve. For studies with inhibitors, DUBs were pre-incubated with derivatives (10-fold molar excess) for 30 minutes before residual Ub-AMC activity was measured.
**SUMO-1 Vinyl Sulfone, human recombinant**  
UL-702  50 µg  
A potent, irreversible and specific inhibitor of SUMO-specific isopeptidases (SENPs). Useful for inhibiting the hydrolysis of poly-SUMO chains on substrate proteins in vitro and thus enhances poly-SUMO chain accumulation.  
**Purity:** ≥98% (HPLC)  
**MW:** ~11.1 kDa

**HA-SUMO-1 Vinyl Sulfone, human recombinant**  
UL-703  50 µg  
This N-terminal HA-tagged SUMO is a potent, irreversible and specific inhibitor of SUMO-specific isopeptidases (SENPs). This protein inhibits the hydrolysis of poly-SUMO chains on substrate proteins in vitro and thus enhances poly-SUMO chain accumulation. The HA peptide sequence (YPYDVPDYA) is derived from the influenza hemagglutinin protein. This epitope allows for the sensitive identification or purification of SENP activities since it is specifically recognized by anti-HA antibodies and/or anti-HA-agarose.  
**Purity:** ≥90% (HPLC)  
**MW:** ~11.1 kDa

**HA-SUMO-3 Vinyl Sulfone, human recombinant**  
UL-769  50 µg  
This N-terminal HA-tagged SUMO is a potent, irreversible and specific inhibitor of SUMO-specific isopeptidases (SENPs). This protein inhibits the hydrolysis of poly-SUMO chains on substrate proteins in vitro and thus enhances poly-SUMO chain accumulation. The HA peptide sequence (YPYDVPDYA) is derived from the influenza hemagglutinin protein. This epitope allows for the sensitive identification or purification of SENP activities since it is specifically recognized by anti-HA antibodies and/or anti-HA-agarose.  
**Purity:** ≥90% (HPLC)  
**MW:** ~10.5 kDa

**NEDD8 Aldehyde, human recombinant**  
UL-801  50 µg  
A potent and highly specific inhibitor of NEDD8-specific isopeptidases (SENPs). This protein blocks the hydrolysis of poly-NEDD8 chains on substrate proteins in vitro and thus enhances poly-NEDD8 chain accumulation.  
**Purity:** ≥98% (HPLC)  
**MW:** ~9.1 kDa

**NEDD8 Vinyl Sulfone, human recombinant**  
UL-802  50 µg  
A potent, irreversible and specific inhibitor of NEDD8-specific isopeptidases (like NEDP1 or SENP8). Useful for inhibiting the hydrolysis of poly-NEDD8 chains on substrate proteins in vitro and thus enhances poly-NEDD8 chain accumulation.  
**Purity:** ≥98% (HPLC)  
**MW:** ~9.1 kDa

**FLAG-NEDD8 Vinyl Sulfone, human recombinant**  
UL-803  25 µg  
This fully functional N-terminally tagged NEDD8 is a potent, irreversible inhibitor of NEDD8-specific isopeptidases (like NEDP1 or SENP8, Catalog # E-800). Useful for inhibiting the hydrolysis of poly-NEDD8 chains on substrate proteins in vitro and thus enhances poly-NEDD8 chain accumulation. The FLAG epitope, allows for the sensitive identification or purification of NEDD8 deconjugating activities since it is specifically recognized by anti-FLAG antibodies M1, M2 or M5.  
**Purity:** ≥90% (HPLC)  
**MW:** ~9.8 kDa

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**Deconjugating Enzyme Antibodies**

**AMSH/STAMBP**  
AF5650  100 µg  
**Species:** Human  
**Form:** Antigen affinity purified goat polyclonal  
**Application(s):** Western blot

**ATG4A**  
AF4324  100 µg  
**Species:** Human  
**Form:** Antigen affinity purified sheep polyclonal  
**Application(s):** Western blot, Immunoprecipitation

**ATG4B/Apg4b**  
MAB4324  100 µg  
**Species:** Human  
**Description:** Monoclonal mouse IgG, Clone # 514914  
**Application(s):** Western blot, Immunoprecipitation

**SENP1 (SUMO1-specific Peptidase 1)**  
AF6587  100 µL  
**Species:** Human  
**Form:** Antigen affinity purified sheep polyclonal  
**Application(s):** Western blot, Immunoprecipitation

**UCH-L1 (Ubiquitin C-terminal Hydrolase L1)**  
A-340  200 µL  
**Species:** Human  
**Form:** Protein A affinity purified rabbit polyclonal  
**Application(s):** Western blot

**AF6007  100 µg**  
**Species:** Human/Mouse/Rat  
**Form:** Antigen affinity purified sheep polyclonal  
**Application(s):** Western blot

**MAB6007  100 µg**  
**Species:** Human/Mouse  
**Description:** Monoclonal mouse IgG, Clone # 671108  
**Application(s):** Western blot, Immunohistochemistry, Immunoprecipitation
### UCH-L3

<table>
<thead>
<tr>
<th>AF6008</th>
<th>100 µg</th>
</tr>
</thead>
</table>

**Species:** Human/Mouse/Rat  
**Form:** Antigen affinity purified sheep polyclonal  
**Application(s):** Western blot

### Usp2 (Ubiquitin Carboxy-terminal Hydrolase 2)

<table>
<thead>
<tr>
<th>AF5804</th>
<th>100 µg</th>
</tr>
</thead>
</table>

**Species:** Mouse  
**Form:** Antigen affinity purified goat polyclonal  
**Application(s):** Western blot

*For a full listing of antibodies, please see pages 89-90.*
Proteasomes are distributed throughout eukaryotic cells at a high concentration and are the primary sites for protein degradation in mammalian cells. Substrate proteins linked to poly-ubiquitin chains are recognized for proteolytic degradation by the proteasome and recycling of ubiquitin monomers by deubiquitinating enzymes.

The 26S proteasome (~2500 kDa) is a multicatalytic enzyme with a highly ordered structure composed of at least 32 different subunits arranged in two sub-complexes (a 20S core and a 19S regulator). The 20S core (700 kDa) is composed of 4 rings of 28 non-identical subunits (2 rings are composed of 7 α subunits and 2 rings are composed of 7 β subunits). Proteolysis occurs within the hollow 20S core which can hydrolyze small peptide substrates, short and unstructured polypeptides and occasionally some proteins with hydrophobic or misfolded patches in vitro. Purified 20S has latent peptidase activity which can be activated by the addition of low amounts of SDS, the attachment of PA28 (and other 11S-Reg type complexes) or the attachment of the 19S regulatory particle. The proteolytic activity of the 20S proteasome can be determined in vitro by using a number of small fluorescent peptide substrates and can be inhibited by various natural products and peptide inhibitors.

The 19S regulator is composed of a base (containing 6 ATPase and 2 non-ATPase subunits) and a lid (containing up to 10 non-ATPase subunits). This regulatory particle is required for the degradation of poly-ubiquitinated substrates. Subunits in the 19S collectively contain ubiquitin receptors, deubiquitinating and ATPase activities. This particle recognizes ubiquitin chains, binds to the substrate, unravels its tertiary structure, aids in translocating it through a gated channel into the 20S core and also disassembles poly-ubiquitin chains.

There also exists a modified form of the proteasome (immunoproteasome) that has the essential function of processing class I MHC peptides. The immunoproteasome contains an alternate regulator, referred to as the 11S regulator (PA28) that replaces the 19S regulator. This non-ATPase activator complex enhances the generation of class I binding peptides by altering the cleavage pattern of the proteasome. The PA28 complex is constitutively expressed in antigen-presenting cells, and its expression is up-regulated by interferon-γ.

**20S Proteasome, rabbit**

<table>
<thead>
<tr>
<th>E-350</th>
<th>25 µg</th>
<th>50 µg</th>
</tr>
</thead>
</table>

The 26S proteasome is a multicatalytic proteinase complex with a highly ordered structure composed of 2 complexes: a 20S core and a 19S regulator. This multi-subunit complex selectively degrades intracellular proteins. The 20S catalytic core is composed of 4 rings of 28 non-identical subunits; 2 rings are composed of 7 α subunits and 2 rings are composed of 7 β subunits. This activity degrades a variety of peptide substrates and poly-ubiquitinated proteins with broad specificity in a non-ATP dependent process. The 20S proteasome can be activated chemically by the addition of detergent or by the proteinaceous activator PA28 (Catalog # E-380).

**Purity:** ≥95% (SDS-PAGE); **MW:** ~700 kDa

**20S Proteasome, rat**

<table>
<thead>
<tr>
<th>E-352</th>
<th>50 µg</th>
</tr>
</thead>
</table>

The 26S proteasome is a multicatalytic proteinase complex with a highly ordered structure composed of 2 complexes, a 20S core and a 19S regulator. This multi-subunit complex selectively degrades intracellular proteins. The 20S catalytic core is composed of 4 rings of 28 non-identical subunits; 2 rings are composed of 7 α subunits and 2 rings are composed of 7 β subunits. This activity degrades a variety of peptide substrates and poly-ubiquitinated proteins with broad specificity in a non-ATP dependent process. The 20S proteasome can be activated chemically by the addition of detergent or by the proteinaceous activator PA28. This product has been purified from rat erythrocytes.

**Purity:** ≥95% (SDS-PAGE); **MW:** ~700 kDa

**20S Proteasome, mouse**

<table>
<thead>
<tr>
<th>E-355</th>
<th>50 µg</th>
</tr>
</thead>
</table>

The 26S proteasome is a multicatalytic proteinase complex with a highly ordered structure composed of 2 complexes: a 20S core and a 19S regulator. This multi-subunit complex selectively degrades intracellular proteins. This activity degrades a variety of peptide substrates and poly-ubiquitinated proteins with broad specificity in a non-ATP dependent process. The 20S proteasome can be activated chemically by the addition of detergent or by the proteinaceous activator PA28 (Catalog # E-380) or 19S (PA700) (Catalog # E-366).

**Purity:** ≥95% (SDS-PAGE) **MW:** ~700 kDa
## 20S Proteasome, human

**E-360** 50 µg

The 26S proteasome is a multicatalytic protease complex with a highly ordered structure composed of 2 complexes: a 20S core and a 19S regulator. This multi-subunit complex selectively degrades intracellular proteins. The 20S catalytic core is composed of 4 rings of 28 non-identical subunits; 2 rings are composed of 7 α subunits and 2 rings are composed of 7 β subunits. This activity degrades a variety of peptide substrates and poly-ubiquitinated proteins with broad specificity in a non-ATP dependent process. The 20S proteasome can be activated chemically by the addition of detergent or by the proteinaceous activator PA28. This highly purified 20S (PA700) proteasome preparation (from transformed HEK cells) can be used in vitro for the activation of 20S proteasome, as well as for deubiquitination of target substrates via associated DUBs, such as RPN11. The 19S subunit is the ATP-dependant regulatory particle (RP) of the 26S proteasome (Catalog # E-365), composed also of a 20S core particle (CP) (Catalog #s E-350, E-360, E-370, E-375) that is responsible for the proteolytic degradation of short-lived and abnormal intracellular proteins. The 19S subunit can bind to one or both ends of the 20S core particle (CP), stimulating peptidase activity by modulating conformational changes and/or facilitating the access of substrates to the proteolytic 20S core.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~700 kDa

### 20S Proteasome, canine

**E-358** 50 µg

The 26S proteasome is a multicatalytic protease complex with a highly ordered structure composed of 2 complexes, a 20S core and a 19S regulator. This multi-subunit complex selectively degrades intracellular proteins. The 20S catalytic core is composed of 4 rings of 28 non-identical subunits; 2 rings are composed of 7 α subunits and 2 rings are composed of 7 β subunits. This activity degrades a variety of peptide substrates and poly-ubiquitinated proteins with broad specificity in a non-ATP dependent process. The 20S proteasome can be activated chemically by the addition of detergent or by the proteinaceous activator PA28. This product has been purified from canine erythrocytes.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~700 kDa

### 26S Proteasome, human

**E-365** 25 µg 50 µg

This highly purified 26S proteasome preparation (from transformed HEK cells) can be used in vitro for the degradation of peptide substrates and poly-ubiquitinated proteins. The 26S proteasome is the major non-lysosomal protease in eukaryotic cells principally responsible for the degradation of ubiquitinated substrates with broad specificity and thus plays a critical role in the regulation of various biological processes. This multicatalytic protease has a highly ordered structure composed of two sub-complexes: a 20S core particle (CP) and a 19S regulatory particle (RP). The 20S (Catalog #s E-350, E-360, E-370, E-375) forms the proteolytic core containing peptidase activities, and the 19S confers ATP-dependency and ubiquitinated substrate specificity. The 19S (PA700) (Catalog # E-366) subunits can bind to one or both ends of the 20S, stimulating peptidase activity by modulating conformational changes and/or facilitating the access of substrates to the 20S core.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~2500 kDa

## 19S Proteasome, human

**E-366** 25 µg

This highly purified 19S (PA700) proteasome preparation (from transformed HEK cells) can be used in vitro for the activation of 20S proteasome, as well as for deubiquitination of target substrates via associated DUBs, such as RPN11. The 19S subunit is the ATP-dependant regulatory particle (RP) of the 26S proteasome (Catalog # E-365), composed also of a 20S core particle (CP) (Catalog #s E-350, E-360, E-370, E-375) that is responsible for the proteolytic degradation of short-lived and abnormal intracellular proteins. The 19S subunit can bind to one or both ends of the 20S core particle (CP), stimulating peptidase activity by modulating conformational changes and/or facilitating the access of substrates to the proteolytic 20S core.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~700 kDa

### 20S Immunoproteasome, human

**E-370** 25 µg

The 20S catalytic core is composed of 4 rings of 28 non-identical subunits; 2 rings are composed of 7 α subunits and 2 rings are composed of 7 β subunits. Immunoproteasomes are modified forms of the constitutive 20S that function in MHC Class I antigen processing. During immunoproteasome assembly, the constitutive catalytic subunits (Delta, Z and X) are replaced by the inducible subunits LMP2, LMP7 and MECL, the former two being encoded by MHC. This protein has been purified from human peripheral blood monocytes, which have been screened and are negative for hepatitis B surface antigen, antibodies to hepatitis C virus, HIV type 1 antigens and antibodies to HIV type 1 and 2.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~700 kDa

### 26S Immunoproteasome, canine

**E-377** 25 µg

The 20S catalytic core is composed of 4 rings of 28 non-identical subunits; 2 rings are composed of 7 α subunits and 2 rings are composed of 7 β subunits. Immunoproteasomes are modified forms of the constitutive 20S that function in MHC Class I antigen processing. The immunoproteasome has undergone an additional modification in which the constitutive catalytic subunits (Delta, Z and X) are replaced by the inducible subunits LMP2, LMP7 and MECL, the former two being encoded by MHC. This protein has been purified from canine spleen.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~700 kDa

### 20S Immunoproteasome, rat

**E-375** 50 µg

The 20S catalytic core is composed of 4 rings of 28 non-identical subunits; 2 rings are composed of 7 α subunits and 2 rings are composed of 7 β subunits. Immunoproteasomes are modified forms of the constitutive 20S that function in MHC Class I antigen processing. The immunoproteasome has undergone an additional modification in which the constitutive catalytic subunits (Delta, Z and X) are replaced by the inducible subunits LMP2, LMP7 and MECL, the former two being encoded by MHC. This protein has been purified from rat spleen.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~700 kDa
20S Immunoproteasome, *mouse*

E-376 50 µg

The 20S catalytic core is composed of 4 rings of 28 non-identical subunits; 2 rings are composed of 7 α subunits and 2 rings are composed of 7 β subunits. Immunoproteasomes are modified forms of the constitutive 20S that function in MHC Class I antigen processing. During immunoproteasome assembly, the constitutive catalytic subunits (Delta, Z and X) are replaced by the inducible subunits LMP2, LMP7 and MECL, the former two being encoded by MHC. This protein has been purified from mouse spleen.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~700 kDa

**PA28 Activator α subunit, human recombinant**

E-380 100 µg

PA28 is a ring-shaped 11S (180 kDa) multimeric complex that can bind to the two ends of the 20S proteasome and dramatically stimulates its capacity to hydrolyze small peptides (but not ubiquitinated proteins or large substrates). In mammals, PA28 is composed of two homologous subunits: PA28α and PA28β, both of which are interferon-induced. *In vitro*, PA28α forms a heptameric ring that can stimulate peptide hydrolysis by 20S particles to the same extent as the heteromeric (3α3β) complex.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~180 kDa

**PA28 Activator β subunit, human recombinant**

E-382 100 µg

PA28 is a ring-shaped 11S multimeric complex that can bind to the two ends of the 20S proteasome and dramatically stimulates its capacity to hydrolyze small peptides (but not ubiquitinated proteins or large substrates). It functions in immunoproteasome assembly, is required for antigen processing and enhances the generation of class I peptides. In mammals, PA28 is composed of two homologous subunits: PA28α and PA28β, both of which are interferon-induced. This protein has been purified from *E.coli*.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~180 kDa

**PA28 Activator γ subunit, human recombinant**

E-384 100 µg

PA28 is a ring-shaped 11S multimeric complex that can bind to the two ends of the 20S proteasome and dramatically stimulates its capacity to hydrolyze small peptides (but not ubiquitinated proteins or large substrates). It functions in immunoproteasome assembly, is required for antigen processing and enhances the generation of class I peptides. Three subunits (α, β and γ) of the 11S regulator have been identified. Six γ subunits combine to form a homo-hexameric ring. This protein has been purified from *E.coli*.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~180 kDa

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### Proteasome Enzyme Antibodies

**S5a/Angiocidin**

AF5540 100 µL

**Species:** Mouse  
**Form:** Antigen affinity purified goat polyclonal  
**Application(s):** Western blot

*For a full listing of antibodies, please see pages 89-90.*
### Inhibitors

**N-Acetyl-L-leucyl-L-leucyl-L-methional**

| 384 | 10 mg | 50 mg |

Peptide aldehydes form a covalent hemiacetal adduct with the 20S proteasome, inhibiting the chymotrypsin-like peptidase activity. These inhibitors are reversible and cell-permeable, and also inhibit the activity of calpains and cathepsins.

**Purity:** >95% (HPLC/TLC). Structure confirmed by NMR

**MW:** 401.6 Da

**Z-Leu-Leu-Leu-CHO (MG-132)**

| 1748 | 1 mg | 5 mg |

Peptide aldehydes form a covalent hemiacetal adduct with the 20S proteasome, inhibiting the chymotrypsin-like peptidase activity. These inhibitors are reversible and cell-permeable, and also inhibit the activity of calpains and cathepsins. Activity tested vs. SDS-activated 20S (Kᵢ = 4.2 nM). Reported IC₅₀ in cell culture is 5 µM.

**Purity:** >95% (HPLC)

**MW:** 475.6 Da

**Lactacystin**

| 2287 | 200 µg |
| 1-155 | 500 µg |
| 1-116 | 1 mg |

Potent, highly specific, irreversible and cell-permeable inhibitor that covalently modifies the catalytic β subunits (chymotrypsin, trypsin- and caspase-like activities) of the proteasome. Lactacystin is hydrolyzed in aqueous solutions to a clasto-lactacystin β-lactone intermediate which is the active inhibitory species that reacts with the proteasome.

**Purity:** ≥95% (HPLC). Structure confirmed by ¹H-NMR

**MW:** 376.4 Da

**AM 114**

| 2564 | 10 mg | 50 mg |


**Purity:** >98% (HPLC).

**MW:** ~377.01 kDa

**Gliotoxin**

| 2637 | 1 mg |

Immunosuppressive agent; blocks phagocytosis, cytokine production and proliferation of T and B cells. Non-competitively inhibits chymotrypsin-like activity of 20S proteasome; prevents degradation of IκBα, an endogenous blocker of NF-κB. Also inhibits farnesyltransferase and geranylgeranyltransferase I (IC₅₀ values are 80 and 17 µM respectively) and displays antitumor activity against breast cancer in vivo.

**FW:** 326.38

**MG-101, Calpain Inhibitor-1**

| 3558 | 5 mg |

Peptide aldehydes form a covalent hemiacetal adduct with the 20S proteasome, inhibiting the chymotrypsin-like peptidase activity. These inhibitors are reversible and cell-permeable, and also inhibit the activity of calpains and cathepsins.

**Purity:** >95% (TLC/HPLC). Structure confirmed by NMR

**MW:** 401.6 Da

**PSI**

| 4045 | 5 mg |

Peptide aldehydes form a covalent hemiacetal adduct with the 20S proteasome, inhibiting the chymotrypsin-like peptidase activity. These inhibitors are reversible and cell-permeable, and also inhibit the activity of calpains and cathepsins.

**Purity:** >95% (HPLC)

**clasto-Lactacystin β-lactone**

| I-100 | 200 µg |
| I-102 | 1 mg |

Potent, highly specific, irreversible and cell-permeable inhibitor that covalently modifies the catalytic β subunits of the proteasome. The β-lactone intermediate is generated from the aqueous hydrolysis of lactacystin and is the active inhibitory species that reacts with the proteasome.

**Purity:** >95% (HPLC).

**MW:** 213.2 Da

**Epoxomicin**

| I-110 | 200 µg |
| I-112 | 1 mg |

Cell-permeable, potent, selective and irreversible proteasome inhibitor initially isolated as natural product from an Actinomycetes strain. Predominantly inhibits the chymotrypsin-like activity of the proteasome.

**Purity:** >95% (HPLC).

**MW:** 554.7 Da

**Z-Leu-Leu-Leu-B(OH)₂ (MG-262)**

| I-120 | 200 µg |
| I-122 | 1 mg |

This peptide boronic acid is structurally similar to the peptide aldehyde MG-132 and also inhibits the chymotrypsin-like peptidase activity of the proteasome but with much higher potency. These inhibitors are reversible and cell-permeable, and also inhibit the activity of calpains and cathepsins. Kᵢ = 0.023 nM vs. SDS-activated 20S proteasome.

**Purity:** >95% (HPLC).

**MW:** 491.4 Da

**Z-Leu-Leu-Nva-CHO (MG-115)**

| I-135 | 5 mg |

Peptide aldehydes form a covalent hemiacetal adduct with the 20S proteasome, inhibiting the chymotrypsin-like peptidase activity. These inhibitors are reversible and cell-permeable, and also inhibit the activity of calpains and cathepsins. Activity tested vs. SDS-activated 20S proteasome (Kᵢ = 20 nM).

**Purity:** >95% (HPLC).

**MW:** 461.6 Da
**Substrates**

**Z-Leu-Leu-Leu-AMC (Z-LLL-AMC)**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>MW</th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-220</td>
<td>642.76 kDa</td>
<td>&gt;95% (TLC/HPLC)</td>
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</tbody>
</table>

Fluorogenic substrate for measuring the chymotrypsin-like peptidase activity of the 20S proteasome. The 20S complex is composed of 28 subunits, arranged in an α,β,β,α, stoichiometry. Each of the two internal β-type rings harbors three different proteolytically active sites, provided by the N-terminal residues of three constitutive subunits: β1 (post-glutamyl peptide hydrolase site), β2 (trypsin-like site) and β5 (chymotrypsin-like site).

**Purity:** >95% (TLC/HPLC). Structure confirmed by NMR MW: 648.8 kDa

**Z-Leu-Leu-Glu-AMC (Z-LLE-AMC)**

<table>
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<th>Substrate</th>
<th>MW</th>
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</thead>
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<tr>
<td>S-230</td>
<td>763.9 kDa</td>
<td>&gt;95% (TLC/HPLC)</td>
</tr>
</tbody>
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Fluorogenic substrate for measuring the chymotrypsin-like peptidase activity of the 20S proteasome. The 20S complex is composed of 28 subunits, arranged in an α,β,β,α, stoichiometry. Each of the two internal β-type rings harbors three different proteolytically active sites, provided by the N-terminal residues of three constitutive subunits: β1 (post-glutamyl peptide hydrolase site), β2 (trypsin-like site) and β5 (chymotrypsin-like site).

**Purity:** >95% (TLC/HPLC). Structure confirmed by NMR MW: 664.8 kDa

**Suc-Leu-Tyr-AMC (Suc-LY-AMC)**

<table>
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<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-260</td>
<td>773.76 kDa</td>
<td>&gt;99% (TLC)</td>
</tr>
</tbody>
</table>

Fluorogenic substrate for measuring the trypsin-like peptidase activity of the 20S proteasome. Each of the two 20S internal β-type rings harbors three different proteolytically active sites: β1 (“post-glutamyl peptide hydrolase” site), β2 (“trypsin-like” site) and β5 (“chymotrypsin-like” site). This peptide is also substrate for the Kex2 endopeptidase from S. cerevisiae, which has substrate specificity toward the C-terminal side of LR, PR and RR sequences. In addition, the peptide can be cleaved by Kallikrein 5 with a P1 site preference for basic residues (R and K).

**Purity:** > 99% (TLC) MW: 773.76 kDa
Conjugation Control Proteins and Peptides

Biotin-Lysozyme Conjugation Substrate, hen egg white

SP-100 50 µg

Lysozyme has been used extensively as a substrate to study the ubiquitin-dependent proteolytic pathway in vitro. This protein is an N-end rule substrate, since the recognition of protein requires the binding of an E3 ligase to the N-terminal lysine residue. Ubiquitin-lysozyme conjugates can be formed in the presence of ATP, ubiquitin and the appropriate conjugation enzyme source. This substrate protein can be used as a positive control with crude fractions (Catalog #s F-340, F-360, F-370, F-372) and conjugation kits (Catalog #s K-915, K-930, K-935, K-960). Additionally, this lysozyme is labeled with biotin for the sensitive detection with avidin affinity reagents, but has all available lysines present for the efficient conjugation to ubiquitin. Isolated from hen egg white.

Purity: ≥95% (SDS-PAGE) MW: ~14 kDa

UBE2K/E2-25K, human recombinant

SP-200 50 µg

This protein can be used as a positive control for SUMOylation assays. E2-25K is modified by SUMO-1, SUMO-2 and SUMO-3 and can be detected on Western blots using anti-E2-25K (Catalog # A-603) or anti-SUMO (Catalog # A-712) antibodies. E2-25K is a class II ubiquitin-conjugating E2 enzyme identified as a target for SUMOylation during a screen for novel SUMO-1 targets. The SUMO modification of E2-25K has been extensively characterized in vitro. This substrate is efficiently SUMOylated by the SUMO activating E1 (SAE1/SAE2) and UbcH9 conjugating enzymes. The biotin group allows for convenient and sensitive detection with avidin-linked reagents. SUMO modification of proteins occurs on lysine residues generally found within a short consensus sequence containing the ψKXE motif. This motif consists of ψ which represents a large hydrophobic amino acid (isoleucine, leucine or valine), K is the lysine that becomes modified, X is any residue and E is glutamic acid. The glutamic acid is the most highly conserved position other than the lysine.

Purity: ≥96% (HPLC) MW: ~1.53 kDa (11 residues)

SUMO Conjugation Peptide Substrate, biotinylated

SP-300 20 µg

This biotinylated peptide contains a SUMOylation consensus sequence and can be covalently conjugated to SUMO-1, SUMO-2 and SUMO-3 proteins in the presence of the E1 activating enzyme (SAE1/SAE2) and UbcH9 conjugating enzymes. The biotin group allows for sensitive and selective detection with avidin-linked reagents. SUMO modification of proteins occurs on lysine residues generally found within a short consensus sequence containing the ψKXE motif. This motif consists of ψ which represents a large hydrophobic amino acid (isoleucine, leucine or valine), K is the lysine that becomes modified, X is any residue and E is glutamic acid. The glutamic acid is the most highly conserved position other than the lysine.

Purity: ≥90% (HPLC) MW: ~1.53 kDa (11 residues)

SUMO Conjugation Negative Control Peptide Substrate, biotinylated

SP-305 20 µg

This biotinylated peptide serves as a negative control and contains a scrambled version of the consensus motif sequence (Catalog # SP-300). It will not be conjugated to SUMO-1, SUMO-2 and SUMO-3 proteins in the presence of the E1 activating (SAE1/SAE2) and UbcH9 conjugating enzymes. The biotin group allows for sensitive and convenient detection using avidin-linked reagents. SUMO modification of proteins occurs on lysine residues generally found within a short consensus sequence containing the ψKXE motif. This motif consists of ψ which represents a large hydrophobic amino acid (isoleucine, leucine or valine), K is the lysine that becomes modified, X is any residue and E is glutamic acid. The glutamic acid is the most highly conserved position other than the lysine.

Purity: ≥90% (HPLC) MW: ~1.53 kDa (11 residues)

**His<sub>6</sub>-S5a/Angiocidin, human recombinant**

SP-400 100 µg

S5a, a component of the 19S complex, functions as a receptor by binding to and recognizing poly-Ub proteins destined for 26S degradation. The protein recognizes Ub chains and protein conjugates via two UIM domains. S5a is a “universal” substrate to monitor E3 activity, since it can be ubiquitinated by a variety of enzymes from different classes. The mechanism of S5a ubiquitination depends on association with Ub or chains via its UIM domains, which brings it into proximity of E3 allowing for modification. The recognition of S5a by E3s is thus atypical and non-selective.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~43 kDa

**S5a/Angiocidin, biotinylated, human recombinant**

SP-405 250 µg

S5a, a component of the 19S complex, functions as a receptor by binding to and recognizing poly-Ub proteins destined for 26S degradation. The protein recognizes Ub chains and protein conjugates via two UIM domains. S5a is a “universal” substrate to monitor E3 activity, since it can be ubiquitinated by a variety of enzymes from different classes. The mechanism of S5a ubiquitination depends on association with Ub or chains via UIM domains, which brings it into proximity of E3 allowing for modification. The recognition of S5a by E3s is thus atypical and non-selective. This protein has been modified with biotin for ease of detection with avidin reagents.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~43 kDa

**His<sub>6</sub>-p53, human recombinant**

SP-450 20 µg

Tumor suppressor protein p53, a nuclear transcription factor, plays an essential role in the regulation of cell cycle and is frequently mutated or inactivated in cancers. Numerous post-translational modifications modulate p53 activity including phosphorylation, acetylation, methylation and ubiquitination. The stability of p53 is regulated via the UPP. MDM2 is an oncogenic E3 that ubiquitinates p53, inhibits its transcriptional activity and promotes degradation. Other E3 ligases that promote the degradation of p53 include Pirh2, COP1 and p300. USP7 stabilizes p53 by deubiquitination and induces p53-dependent cell growth repression and apoptosis. Additional factors such as p14ARF and MdmX also modulate p53 function via the UPP. Accession # NP_000537.

**Purity:** ≥90% (SDS-PAGE)  **MW:** ~45.6 kDa

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**SUMOylated peptide**

Western blot detection of consensus peptide (SP-300) modification by SUMO proteins (UL-712, UL-752, UL-762) using SUMO Conjugation kits (Catalog #s K-710, K-715, K-720).

**Modification of E2-25K (SP-200) using SUMO-1 Conjugation Kit (Catalog # K-710), SUMO-2 Conjugation Kit (Catalog # K-715), or SUMO-3 Conjugation Kit (Catalog # K-720).**

**Deubiquitination of p53 by USP7 and USP8.** Poly-ubiquitinated p53 was prepared as described in figure above, and incubated with increasing amounts of USP7 (Catalog # E-518) and USP8 (Catalog # E-520). Reactions were incubated at 37 °C for 1 hour. Western blotting with a p53-specific monoclonal antibody was performed. The lower panel shows a shorter exposure to indicate the appearance of p53 after chain removal.
### Substrate Protein Antibodies

#### p53

- **AF1355** 100 µg  
  **Species:** Human/Mouse/Rat  
  **Form:** Antigen affinity purified goat polyclonal  
  **Application(s):** Western blot, Chromatin Immunoprecipitation

- **BAF1355** 50 µg  
  **Species:** Human/Mouse/Rat  
  **Form:** Biotinylated antigen affinity purified goat polyclonal  
  **Application(s):** Western blot

- **GAF1355** 250 µL  
  **Species:** Human/Mouse/Rat  
  **Form:** Agarose-conjugated affinity purified goat polyclonal  
  **Application(s):** Immunoprecipitation

- **HAF1355** 100 µL  
  **Species:** Human/Mouse/Rat  
  **Form:** HRP-conjugated antigen affinity purified goat polyclonal  
  **Application(s):** Western blot

- **MAB1355** 100 µg  
  **Species:** Human/Mouse/Rat  
  **Form:** Monoclonal mouse IgG2B, Clone # 184721  
  **Application(s):** Western blot, Immunoprecipitation

- **MAB13551** 100 µg  
  **Species:** Human  
  **Form:** Monoclonal mouse IgG2B, Clone # 184727  
  **Application(s):** Intracellular Staining by Flow Cytometry

- **IC13551A** 100 Tests  
  **Species:** Human  
  **Form:** Allophycocyanin-conjugated monoclonal mouse IgG1, Clone # 184727  
  **Application(s):** Flow Cytometry

#### Phospho-p53 (S15) continued

- **MAB1839** 100 µg  
  **Species:** Human  
  **Form:** Monoclonal mouse IgG1, Clone # 261352  
  **Application(s):** Western blot, Intracellular Staining by Flow Cytometry

- **IC1839A** 100 Tests  
  **Species:** Human  
  **Form:** Allophycocyanin-conjugated monoclonal mouse IgG1, Clone # 261352  
  **Application(s):** Flow Cytometry

- **IC1839P** 100 Tests  
  **Species:** Human  
  **Form:** Phycoerythrin-conjugated monoclonal mouse IgG1, Clone # 261352  
  **Application(s):** Flow Cytometry

#### Phospho-p53 (S18)

- **AF2887** 100 µg  
  **Species:** Mouse  
  **Form:** Antigen affinity purified rabbit polyclonal  
  **Application(s):** Western blot

#### Phospho-p53 (S20)

- **AF2286** 100 µg  
  **Species:** Human  
  **Form:** Antigen affinity purified rabbit polyclonal  
  **Application(s):** Western blot

#### Phospho-p53 (S37)

- **AF3306** 50 µg  
  **Species:** Human  
  **Form:** Antigen affinity purified rabbit polyclonal  
  **Application(s):** Western blot

#### Phospho-p53 (S46)

- **AF1489** 100 µg  
  **Species:** Human  
  **Form:** Antigen affinity purified rabbit polyclonal  
  **Application(s):** Western blot, Immunohistochemistry, Immunoprecipitation
Phospho-p53 (S392)
AF2996 50 µg
Species: Human
Form: Antigen affinity purified rabbit polyclonal
Application(s): Western blot

UBE2K/E2-25K
AF6609 100 µg
Species: Human/Mouse/Rat
Form: Antigen affinity purified sheep polyclonal
Application(s): Western blot

MAB6609 100 µg
Species: Human/Mouse
Form: Monoclonal mouse IgG2B, Clone # 701316
Application(s): Western blot, Immunohistochemistry

For a full listing of antibodies, please see pages 89-90.

Substrate Protein ELISA Kits

Total p53
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Species: Human

Phospho-p53 (S15)
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Species: Human

Phospho-p53 (S46)
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Species: Human

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Species: Human

Active p53
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<td>DYC1355E</td>
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</table>

Species: Human/Mouse
Ubiquitin and Ubiquitin Derivatives

Ubiquitin is a highly conserved globular 76-residue eukaryotic protein found in the cytoplasm and nuclei of cells. Ubiquitin exists both as a monomer and as isopeptide-linked polymers known as poly-ubiquitin chains. Ubiquitin modification is required for ATP-dependent (non-lysosomal) proteolysis of both abnormal proteins and normal proteins with rapid turnover. Ubiquitin modification is now also implicated in regulating the activity of proteins in non-proteasomal pathways.

The overall process of ubiquitin-dependent metabolism is a multi-enzymatic process requiring the successive activities of distinct conjugating (E1s, E2s and E3s) and deubiquitinating enzymes (DUBs). Much of the chemistry of ubiquitin conjugation is confined to the conformationally flexible and accessible C-terminal Gly75-Gly76 residues, and the seven internal lysine residues which are critical for the formation of poly-ubiquitin chains. Chemical modification of ubiquitin protein results in several useful derivatives. Modifying the C-terminal glycine carboxyl of ubiquitin to an aldehyde results in a highly potent inhibitor of DUBs. Alternatively, a fluorogenic substrate for DUBs is generated by synthetically conjugating AMC to the C-terminus of ubiquitin. In addition, reductive methylation of the amine groups prevents the formation of poly-ubiquitin chains via lysine linkages. Ubiquitin can also be coupled to agarose via its primary amines, leaving the C-terminus free and available to bind ubiquitin binding proteins.

Ubiquitin

**Ubiquitin, mammalian**

U-100 10 mg

Highly purified ubiquitin processed for the quantitative removal of glycine and buffer salts which can interfere with chemical and in vitro reactions. Ubiquitin is a highly conserved 76 amino acid nuclear and cytoplasmic protein. It is found exclusively in eukaryotes, becomes covalently attached to substrate proteins by enzymes in the Ubiquitin-Proteasome Pathway (UPP) and has a major role in targeting cellular proteins for the ATP-dependent degradation by the 26S proteasome. Ubiquitination also affects proteasome-independent events such as protein localization, activity and function.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~8.5 kDa

**Ubiquitin, human recombinant**

U-100H 10 mg

Highly purified ubiquitin processed for the quantitative removal of glycine and buffer salts which can interfere with chemical and in vitro reactions. Ubiquitin is a 76 amino acid highly conserved nuclear and cytoplasmic protein. It is found exclusively in eukaryotes, becomes covalently attached to substrate proteins by enzymes in the Ubiquitin-Proteasome Pathway (UPP) and has a major role in targeting cellular proteins for the ATP-dependent degradation by the 26S proteasome. Ubiquitination also affects proteasome-independent events such as protein localization, activity and function.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~8.5 kDa

**Ubiquitin, yeast recombinant**

U-100Sc 5 mg

Highly purified ubiquitin free of glycine and buffer salts which can interfere with chemical and in vitro reactions. Ubiquitin is a 76 amino acid, highly conserved nuclear and cytoplasmic eukaryotic protein. It is covalently attached to substrate proteins by enzymes in the Ubiquitin-Proteasome Pathway (UPP). The major role of ubiquitination is to target cellular proteins for the ATP-dependent degradation by the 26S proteasome and proteasome-independent or regulatory events such as protein localization, activity and function. This protein sequence is for *S. cerevisiae* (P61864) and is exactly the same for *S. pombe*.

**Purity:** ≥98%  **(HPLC)**  **MW:** ~8.6 kDa

**Ubiquitin, plant recombinant**

U-100At 5 mg

Highly purified ubiquitin free of glycine and buffer salts which can interfere with chemical and in vitro reactions. Ubiquitin is a 76 amino acid, highly conserved nuclear and cytoplasmic eukaryotic protein. It is covalently attached to substrate proteins by enzymes in the Ubiquitin-Proteasome Pathway (UPP). The major role of ubiquitination is to target cellular proteins for the ATP-dependent degradation by the 26S proteasome and proteasome-independent or regulatory events such as protein localization, activity and function. This protein sequence is for *A. thaliana* (P59263) and the sequence is entirely homologous in barley, oat, soybean and other plants.

**Purity:** ≥95%  **(SDS-PAGE)**  **MW:** ~8.6 kDa

HA-Ub-Vinyl Sulfone reacts irreversibly with ubiquitin conjugating enzymes. Enzymes (200 ng) were incubated with or without a 3-fold molar excess HA-Ub-VS in 50 mM Hepes pH 8.0, 150 mM NaCl, 2 mM DDT and incubated at 37 °C for 60 minutes. Proteins were separated by SDS-PAGE (4-20%) and covalent complex formation was detected by Western blotting with an anti-HA polyclonal antibody.
**Ubiquitin, plasmodium recombinant**

**U-100Pf**

Highly purified ubiquitin free of glycine and buffer salts which can interfere with chemical and *in vitro* reactions. Ubiquitin is a 76 amino acid, highly conserved nuclear and cytoplasmic eukaryotic protein. It is covalently attached to substrate proteins by enzymes in the Ubiquitin-Proteasome Pathway (UPP). The major role of ubiquitination is to target cellular proteins for the ATP-dependent degradation by the 26S proteasome and proteasome-independent or regulatory events such as protein localization, activity and function. This protein sequence is for *protozoan parasite P. falciparum* (NP_701482) which causes malaria. Considering its conserved role among eukaryotes, this system is expected to regulate key molecular events driving the parasite life cycle, including parasite discrete apicomplexan mechanisms such as host cell invasion and apicoplast formation.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~9.3 kDa

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### Ubiquitin Derivatives

**HA-Ubiquitin, human recombinant**

**U-110**

This fully functional N-terminal HA-tagged ubiquitin protein allows for the convenient detection or affinity purification of ubiquitinated proteins *in vitro*. The HA peptide sequence (YPYDVPDYA) is an epitope derived from the influenza hemagglutinin protein. This tag is specifically recognized by anti-HA antibodies and anti-HA-agarose. Ubiquitin is a 76 amino acid, highly conserved nuclear and cytoplasmic protein. It is found exclusively in eukaryotes, becomes covalently attached to substrate proteins by enzymes in the Ubiquitin-Proteasome Pathway (UPP) and has a major role in targeting cellular proteins for the ATP-dependent degradation by the 26S proteasome. Ubiquitination also affects proteasome-independent events such as protein localization, activity and function.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~8.6 kDa

**myc-Ubiquitin, human recombinant**

**U-115**

This fully functional N-terminal myc-tagged ubiquitin protein allows for the convenient detection or affinity purification of ubiquitinated proteins *in vitro*. The tag sequence (EQKLISEEDL) is an epitope derived from the C-terminal region of the human proto oncoprotein p62c-myc. This tag is specifically recognized by anti-myc antibodies or anti-myc-agarose. Ubiquitin is a 76 amino acid, highly conserved nuclear and cytoplasmic protein. It is found exclusively in eukaryotes, becomes covalently attached to substrate proteins by enzymes in the Ubiquitin-Proteasome Pathway (UPP) and has a major role in targeting cellular proteins for the ATP-dependent degradation by the 26S proteasome. Ubiquitination also affects proteasome-independent events such as protein localization, activity and function.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~9.3 kDa

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**FLAG (DYKDDDK)-Ubiquitin, human recombinant**

**U-120**

This fully functional N-terminal FLAG-tagged ubiquitin protein allows for the convenient detection or affinity purification of ubiquitinated proteins *in vitro*. The epitope tag is a hydrophilic octapeptide (DYKDDDK) derived from the sequence of the bacteriophage 7 gene-10 product. This tag is specifically recognized by anti-FLAG antibodies M1, M2 or M5. Ubiquitin is a 76 amino acid, highly conserved nuclear and cytoplasmic protein. It is found exclusively in eukaryotes, becomes covalently attached to substrate proteins by enzymes in the Ubiquitin-Proteasome Pathway (UPP) and has a major role in targeting cellular proteins for the ATP-dependent degradation by the 26S proteasome. Ubiquitination also affects proteasome-independent events such as protein localization, activity and function.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~9.3 kDa

**Ubiquitin Aldehyde, human recombinant**

**U-201**

A potent and highly specific inhibitor of all ubiquitin C-terminal hydrolases. This protein blocks the hydrolysis of poly-ubiquitin chains on substrate proteins *in vitro* and thus enhances poly-ubiquitin chain accumulation. Inhibits UCH Isopeptidase-T (Kᵢ = 2.5 nM).

**Purity:** ≥96% (HPLC)  **MW:** ~8.5 kDa

**Ubiquitin Vinyl Sulfone, human recombinant**

**U-202**

A potent, irreversible and specific inhibitor of all ubiquitin C-terminal hydrolases (UCHs), ubiquitin-specific proteases (USPs) and deubiquitinating enzymes (DUBs). Useful for inhibiting the hydrolysis of poly-ubiquitin chains on substrate proteins *in vitro* and thus enhances poly-ubiquitin chain accumulation.

**Purity:** ≥98% (HPLC)  **MW:** ~8.6 kDa

**Ubiquitin Vinyl Methyl Ester, human recombinant**

**U-203**

A potent, irreversible and specific inhibitor of all ubiquitin C-terminal hydrolases (UCHs), ubiquitin-specific proteases (USPs) and deubiquitinating enzymes (DUBs). Useful for inhibiting the hydrolysis of poly-ubiquitin chains on substrate proteins *in vitro* and thus enhances poly-ubiquitin chain accumulation.

**Purity:** ≥98% (HPLC)  **MW:** ~8.6 kDa

**HA-Ubiquitin Aldehyde, human recombinant**

**U-211**

Ubiquitin-aldehyde is a potent and specific inhibitor of most deubiquitinating enzymes (DUBs) such as ubiquitin C-terminal hydrolases (UCHs) and ubiquitin-specific proteases (USPs). It prevents the hydrolysis of poly-ubiquitin chains on substrate proteins *in vitro* and thus enhances poly-ubiquitin chain accumulation. This tagged version contains an N-terminal HA peptide sequence (YPYDVPDYA) derived from the influenza hemagglutinin protein. This epitope allows for the sensitive identification or purification of DUBs since it is specifically recognized by anti-HA antibodies and/or anti-HA-agarose.

**Purity:** ≥95% (HPLC)  **MW:** ~9.5 kDa

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*www.bostonbiochem.com*
HA-Ubiquitin Vinyl Sulfone, human recombinant

U-212 25 µg

This N-terminal HA-tagged ubiquitin is a potent, irreversible and specific inhibitor of most deubiquitinating enzymes (DUBs) including ubiquitin C-terminal hydrolases (UCHs) and ubiquitin-specific proteases (USPs). Useful for inhibiting the hydrolysis of poly-ubiquitin chains on substrate proteins in vitro and thus enhances poly-ubiquitin chain accumulation. The HA peptide sequence (YPYDVPDYA) is derived from the influenza hemagglutinin protein. This epitope allows for the sensitive identification or purification of DUBs since it is specifically recognized by anti-HA antibodies and/or anti-HA-agarose.

Purity: ≥95% (HPLC)  MW: ~9.5 kDa

Ubiquitin Agarose, human recombinant

U-405 0.5 mL
U-400 1 mL

Ubiquitin is covalently coupled to agarose beads via primary amines allowing for a fully functional C-term. Useful for affinity binding of ubiquitin activating enzyme (E1), ubiquitin carrier enzymes (E2s), ubiquitin ligases (E3s), ubiquitin C-terminal hydrolases (UCHs), and other proteins/enzymes that have an affinity for ubiquitin.

Purity: ≥95% (SDS-PAGE)  MW: ~9.5 kDa

Methylated Ubiquitin, human recombinant

U-502 1 mg
U-501 5 mg

Reductive methylation of lysine residues renders ubiquitin unable to form poly-ubiquitin chains via lysine linkages with other Ub molecules. Methylated Ub can form an E1-catalyzed active thioester at the C-term allowing the molecule to be transferred to the lysines of substrate proteins (which can be mono-ubiquitinated). Ideal for the reduction in poly-ubiquitin chain length and rates of ubiquitin conjugation.

Purity: ≥95% (SDS-PAGE)  MW: ~8.5 kDa

His6-Ubiquitin, human recombinant

U-530 2 mg

Fully functional N-terminal His6-tagged ubiquitin which allows for metal chelate affinity purification of ubiquitinated proteins. Also allows for convenient immunodetection of conjugates using His6-specific antibodies.

Purity: ≥95% (SDS-PAGE)  MW: ~8.5 kDa

GST-Ubiquitin, human recombinant

U-540 1 mg

Fully functional ubiquitin with an N-terminal GST-tag (glutathione S-transferase). GST-Ub allows for glutathione affinity purification of ubiquitinated molecules and allows for immunodetection of conjugates using GST antibodies. Facilitates the facile visualization of poly-Ub chains due to larger ladder intervals.

Purity: ≥95% (SDS-PAGE)  MW: ~38.5 kDa

Ubiquitin AMC, human recombinant

U-550 50 µg

Fluorogenic substrate for ubiquitin hydrolases based on the C-terminal derivatization of ubiquitin with 7-amido-4-trifluoromethylcoumarin (AMC). Ubiquitin-AMC is an exquisitely sensitive substrate for UCH-L3 (K_m = 0.039 µM) and for the Isopeptidase-T (K_m = 0.17 - 1.4 µM). Ub-AMC is useful for studying ubiquitin hydrolases when detection sensitivity or continuous monitoring of activity is essential.

Purity: ≥95% (HPLC)  MW: ~8.6 kDa

Ubiquitin AFC, human recombinant

U-551 50 µg

Fluorogenic substrate based on the C-terminal derivatization of ubiquitin with 7-amino-4-trifluoromethylcoumarin (AFC). Similar to ubiquitin AMC, this is an exquisitely sensitive deubiquitinating enzyme substrate and is useful for studying ubiquitin C-terminal hydrolytic activity when detection sensitivity or continuous monitoring is essential. The fluorophore has a larger Stokes radius than AMC which is useful to reduce compound interference in HTS assays.

Purity: ≥95% (HPLC)  MW: ~8.6 kDa

Ubiquitin-Rhodamine110 (R110), human recombinant

U-555 50 µg

Fluorogenic substrate for deubiquitinating enzymes based on the C-terminal derivative of ubiquitin with Rhodamine110 (R110). Similar to other C-term derivatives such as Ub-AMC (Catalog # U-550) and Ub-AFC (Catalog # U-551), this is an exquisitely sensitive deubiquitinating enzyme substrate and is useful for studying ubiquitin C-terminal hydrolytic activity when detection sensitivity or continuous monitoring of activity at longer wavelengths is essential.

Purity: ≥95% (HPLC)  MW: ~8.5 kDa

Ubiquitin-Aminoluciferin (AML), human recombinant

U-556 50 µg

Substrate for deubiquitinating enzymes based on the C-terminal derivative of ubiquitin with aminoluciferin (AML). Rather than fluorescence as the indicator of DUB activity, DUB liberated luciferin is processed by luciferase to give a luminescence signal. Ub-AML not only produces a stronger signal, but also has an excellent signal to noise ratio over traditional fluorophores. This makes it possible to rapidly assess the activity of DUBs that poorly utilize Ub-AMC while using much lower levels of the DUBs themselves.

Purity: ≥95% (HPLC)  MW: ~8.8 kDa

Ubiquitin-Lys-TAMRA (Ub-Gly-Gly76c-Lys-TAMRA-Gly-OH)

U-558 50 µg

Fluorescence polarization high-throughput screening (HTS) reagent which is based on a 5-tetramethylrhodamine (TAMRA) modified Lys-Gly sequence that is linked to ubiquitin via a native isopeptide bond with the lysine side-chain. This reagent is useful for studying ubiquitin C-terminal hydrolytic activity when detection sensitivity or continuous monitoring of activity at longer wavelengths is essential.

Purity: ≥95% (HPLC)  MW: ~9.2 kDa

Di-Ub (K11-Linked) FRET TAMRA Pos4, human recombinant

UF-440 50 µg

Linkage specific diubiquitin is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. These FRET-based DUB substrates with isopeptide bonds between ubiquitin moieties are superior to gel densitometry assays. Since DUBs recognize and cleave substrates with specific steric conditions, each substrate varies in the position of the fluorophore and quencher and must be empirically tested for individual enzymes. Ubiquitination through K11 is important for endoplasmic reticulum-associated degradation (ERAD) and there undoubtedly exist DUBs that may have specificity for K11 linkages.

Purity: ≥95% (HPLC)  MW: ~17 kDa
Di-Ub (K48-linked) FRET TAMRA Pos1, human recombinant

UF-210 50 µg

Linkage specific diubiquitin is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. These FRET-based DUB substrates with isopeptide bonds between ubiquitin moieties are superior to gel densitometry assays. Since DUBs recognize and cleave substrates with specific steric conditions, each substrate varies in the position of the fluorophore and quencher and must be empirically tested for individual enzymes.

Purity: ≥95% (HPLC)  MW: ~17 kDa

Di-Ub (K48-linked) FRET EDANS Pos1, human recombinant

UF-231 50 µg

Linkage specific diubiquitin is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. These FRET-based DUB substrates with isopeptide bonds between ubiquitin moieties are superior to gel densitometry assays. Since DUBs recognize and cleave substrates with specific steric conditions, each substrate varies in the position of the fluorophore and quencher and must be empirically tested for individual enzymes.

Purity: ≥95% (HPLC)  MW: ~17 kDa

Di-Ub (K63-linked) FRET TAMRA Pos1, human recombinant

UF-311 50 µg

Linkage specific diubiquitin is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. These FRET-based DUB substrates with isopeptide bonds between ubiquitin moieties are superior to gel densitometry assays. Since DUBs recognize and cleave substrates with specific steric conditions, each substrate varies in the position of the fluorophore and quencher and must be empirically tested for individual enzymes.

Purity: ≥95% (HPLC)  MW: ~17 kDa

Di-Ub (K48-linked) FRET TAMRA Pos2, human recombinant

UF-220 50 µg

Linkage specific diubiquitin is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. These FRET-based DUB substrates with isopeptide bonds between ubiquitin moieties are superior to gel densitometry assays. Since DUBs recognize and cleave substrates with specific steric conditions, each substrate varies in the position of the fluorophore and quencher and must be empirically tested for individual enzymes.

Purity: ≥95% (HPLC)  MW: ~17 kDa

Di-Ub (K48-linked) FRET EDANS Pos2, human recombinant

UF-221 50 µg

Linkage specific diubiquitin is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. These FRET-based DUB substrates with isopeptide bonds between ubiquitin moieties are superior to gel densitometry assays. Since DUBs recognize and cleave substrates with specific steric conditions, each substrate varies in the position of the fluorophore and quencher and must be empirically tested for individual enzymes.

Purity: ≥95% (HPLC)  MW: ~17 kDa

Di-Ub (K63-linked) FRET TAMRA Pos2, human recombinant

UF-320 50 µg

Linkage specific diubiquitin is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. These FRET-based DUB substrates with isopeptide bonds between ubiquitin moieties are superior to gel densitometry assays. Since DUBs recognize and cleave substrates with specific steric conditions, each substrate varies in the position of the fluorophore and quencher and must be empirically tested for individual enzymes.

Purity: ≥95% (HPLC)  MW: ~17 kDa

Di-Ub (K48-linked) FRET TAMRA Pos3, human recombinant

UF-230 50 µg

Linkage specific diubiquitin is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. These FRET-based DUB substrates with isopeptide bonds between ubiquitin moieties are superior to gel densitometry assays. Since DUBs recognize and cleave substrates with specific steric conditions, each substrate varies in the position of the fluorophore and quencher and must be empirically tested for individual enzymes.

Purity: ≥95% (HPLC)  MW: ~17 kDa

Di-Ub (K63-linked) FRET TAMRA Pos3, human recombinant

UF-330 50 µg

Linkage specific diubiquitin is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. These FRET-based DUB substrates with isopeptide bonds between ubiquitin moieties are superior to gel densitometry assays. Since DUBs recognize and cleave substrates with specific steric conditions, each substrate varies in the position of the fluorophore and quencher and must be empirically tested for individual enzymes.

Purity: ≥95% (HPLC)  MW: ~17 kDa
### Ubiquitin Antibodies

#### Ubiquitin

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Concentration</th>
<th>Species</th>
<th>Form</th>
<th>Application(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-100</td>
<td>200 µL</td>
<td>Human</td>
<td>Affinity purified rabbit polyclonal</td>
<td>Western blot</td>
</tr>
</tbody>
</table>

#### Ubiquitin+1

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Concentration</th>
<th>Species</th>
<th>Form</th>
<th>Application(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF703</td>
<td>100 µg</td>
<td>Human</td>
<td>Affinity purified rabbit polyclonal</td>
<td>Western blot</td>
</tr>
</tbody>
</table>

For a full listing of antibodies, please see pages 89-90.

### Ubiquitin ELISA Kits

#### Ubiquitin+1

<table>
<thead>
<tr>
<th>Kit Code</th>
<th>Plates</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>DY703-5</td>
<td>5</td>
<td>Human</td>
</tr>
<tr>
<td>DY703E</td>
<td>15</td>
<td>Human</td>
</tr>
</tbody>
</table>

Species: Human
Labeled Ubiquitin Proteins

Ubiquitin proteins modified by biotin or fluorescein are activated by the E1 enzyme and are competent substrates for E2 and E3 enzymes. These labels are useful for the sensitive detection (with avidin affinity reagents or fluorescence) or for the affinity purification of ubiquitin conjugates or ubiquitin binding proteins.

Since the chemistry of ubiquitin conjugation involves its C-terminus and seven lysine residues, the exclusive modification at the N-terminus results in a fully functional molecule.

**Biotin**

**His6-Ubiquitin N-terminal Biotin, human recombinant**

<table>
<thead>
<tr>
<th>Purity: ≥95% (SDS-PAGE)</th>
<th>MW: ~8.6 kDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>UB-HK630</td>
<td>50 µg</td>
</tr>
</tbody>
</table>

Produced via a proprietary process resulting in a ubiquitin modification exclusively on the N-terminus of ubiquitin. This site-specific modification results in an ubiquitin that is fully functional at the C-terminus and with the full complement of reactive lysines to allow for poly-ubiquitin chain incorporation. Detection with avidin-linked reagents allows for a higher efficiency and detection sensitivity than with anti-ubiquitin antibodies. Ideal as an alternative to radio-labeled ubiquitin.

**Ubiquitin Biotin, human recombinant**

<table>
<thead>
<tr>
<th>Purity: ≥95% (SDS-PAGE)</th>
<th>MW: ~8.6 kDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>UB-570</td>
<td>100 µg</td>
</tr>
</tbody>
</table>

Ubiquitin modified with biotin via primary amine coupling. This results in multiple biotinylated ubiquitin species modified at the N-terminus, as well as lysine residues. Although having a fully functional C-terminus, lysine modification may limit the ability of this reagent to propagate poly-ubiquitin chains. Biotinylated ubiquitin can be detected using avidin-linked reagents.

**Ubiquitin N-terminal Biotin, human recombinant**

<table>
<thead>
<tr>
<th>Purity: ≥95% (SDS-PAGE)</th>
<th>MW: ~8.6 kDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>UB-560</td>
<td>50 µg</td>
</tr>
</tbody>
</table>

Produced via a proprietary process resulting in a ubiquitin modification exclusively on the N-terminus of ubiquitin. This site-specific modification results in an ubiquitin that is fully functional at the C-terminus, and with the full complement of reactive lysines to allow for poly-ubiquitin chain incorporation. Detection with avidin-linked reagents allows for a higher efficiency and detection sensitivity than with anti-ubiquitin antibodies. Ideal as an alternative to radio-labeled ubiquitin.

**Ubiquitin K48 only, N-Terminal Biotin, human recombinant**

<table>
<thead>
<tr>
<th>Purity: ≥95% (SDS-PAGE)</th>
<th>MW: ~8.6 kDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>UB-K480</td>
<td>50 µg</td>
</tr>
</tbody>
</table>

Produced via a proprietary process resulting in a single biotin modification exclusively on the N-terminus of ubiquitin. This site-specific modification results in an ubiquitin that is fully functional at the C-terminus, and with the full complement of reactive lysines to allow for poly-ubiquitin chain incorporation. Detection with avidin-linked reagents allows for a higher efficiency and detection sensitivity than with anti-ubiquitin antibodies. Ideal as an alternative to radio-labeled ubiquitin. This ubiquitin mutant contains only a single lysine (K48) with all other lysines mutated to arginine, and is able to form poly-ubiquitin chains with other ubiquitin molecules via the K48 lysine only.

**His6-Ubiquitin K63 only, N-Terminal Biotin, human recombinant**

<table>
<thead>
<tr>
<th>Purity: ≥95% (SDS-PAGE)</th>
<th>MW: ~8.6 kDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>UB-HK630</td>
<td>50 µg</td>
</tr>
</tbody>
</table>

Produced via a proprietary process resulting in a single biotin modification exclusively on the N-terminus of ubiquitin. This site-specific modification results in an ubiquitin that is fully functional at the C-terminus, and with the full complement of reactive lysines to allow for poly-ubiquitin chain incorporation. Detection with avidin-linked reagents allows for a higher efficiency and detection sensitivity than with anti-ubiquitin antibodies. Ideal as an alternative to radio-labeled ubiquitin. This ubiquitin mutant contains only a single lysine (K63) with all other lysines mutated to arginine, and is able to form poly-ubiquitin chains with other ubiquitin molecules via the K63 lysine only.

**Ubiquitin K63 only, N-Terminal Biotin, human recombinant**

<table>
<thead>
<tr>
<th>Purity: ≥95% (SDS-PAGE)</th>
<th>MW: ~8.6 kDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>UB-K630</td>
<td>50 µg</td>
</tr>
</tbody>
</table>

Produced via a proprietary process resulting in a ubiquitin modification exclusively on the N-terminus of ubiquitin. This site-specific modification results in an ubiquitin that is fully functional at the C-terminus, and with the full complement of reactive lysines to allow for poly-ubiquitin chain incorporation. Detection with avidin-linked reagents allows for a higher efficiency and detection sensitivity than with anti-ubiquitin antibodies. Ideal as an alternative to radio-labeled ubiquitin. This ubiquitin mutant contains only a single lysine (K63) with all other lysines mutated to arginine, and is able to form poly-ubiquitin chains with other ubiquitin molecules via the K63 lysine only.

**His6-Ubiquitin K48 only, N-Terminal Biotin, human recombinant**

<table>
<thead>
<tr>
<th>Purity: ≥95% (SDS-PAGE)</th>
<th>MW: ~9.3 kDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>UB-HK480</td>
<td>50 µg</td>
</tr>
</tbody>
</table>

Produced via a proprietary process resulting in a single biotin modification exclusively on the N-terminus of ubiquitin. This site-specific modification results in an ubiquitin that is fully functional at the C-terminus, and with the full complement of reactive lysines to allow for poly-ubiquitin chain incorporation. Detection with avidin-linked reagents allows for a higher efficiency and detection sensitivity than with anti-ubiquitin antibodies. Ideal as an alternative to radio-labeled ubiquitin. This ubiquitin mutant contains only a single lysine (K48) with all other lysines mutated to arginine, and is able to form poly-ubiquitin chains with other ubiquitin molecules via the K48 lysine only.

**Ubiquitin K48 only, N-Terminal Biotin, human recombinant**

<table>
<thead>
<tr>
<th>Purity: ≥95% (SDS-PAGE)</th>
<th>MW: ~9.3 kDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>UB-K480</td>
<td>50 µg</td>
</tr>
</tbody>
</table>

Produced via a proprietary process resulting in a single biotin modification exclusively on the N-terminus of ubiquitin. This site-specific modification results in an ubiquitin that is fully functional at the C-terminus, and with the full complement of reactive lysines to allow for poly-ubiquitin chain incorporation. Detection with avidin-linked reagents allows for a higher efficiency and detection sensitivity than with anti-ubiquitin antibodies. Ideal as an alternative to radio-labeled ubiquitin. This ubiquitin mutant contains only a single lysine (K48) with all other lysines mutated to arginine, and is able to form poly-ubiquitin chains with other ubiquitin molecules via the K48 lysine only.

**His6-Ubiquitin K63 only, N-Terminal Biotin, human recombinant**

<table>
<thead>
<tr>
<th>Purity: ≥95% (SDS-PAGE)</th>
<th>MW: ~9.3 kDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>UB-HK630</td>
<td>50 µg</td>
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</tbody>
</table>

Produced via a proprietary process resulting in a ubiquitin modification exclusively on the N-terminus of ubiquitin. This site-specific modification results in an ubiquitin that is fully functional at the C-terminus, and with the full complement of reactive lysines to allow for poly-ubiquitin chain incorporation. Detection with avidin-linked reagents allows for a higher efficiency and detection sensitivity than with anti-ubiquitin antibodies. Ideal as an alternative to radio-labeled ubiquitin. This ubiquitin mutant contains only a single lysine (K63) with all other lysines mutated to arginine, and is able to form poly-ubiquitin chains with other ubiquitin molecules via the K63 lysine only.

www.bostonbiochem.com
Ubiquitin, No Lysines N-Terminal Biotin, human recombinant

UB-NOK 50 µg

Produced via a proprietary process resulting in a single biotin modification exclusively on the N-terminus of ubiquitin. This site-specific modification results in an ubiquitin that is fully functional at the C-terminus. This ubiquitin mutant lacks all reactive lysine residues which have been mutated to arginine. These mutations render the protein unable to form poly-ubiquitin chains and can be used as a negative control or to detect mono-ubiquitination. Detection with avidin-linked reagents allows for a higher efficiency and detection sensitivity than with anti-ubiquitin antibodies. Ideal as an alternative to radio-labeled ubiquitin.

Purity: ≥95% (SDS-PAGE) MW: ~8.6 kDa

Fluorescein

Ubiquitin, N-Terminal Fluorescein, human recombinant

U-580 50 µg

Produced via a proprietary process resulting in a single fluorescein modification exclusively on the N-terminus of ubiquitin. This site-specific modification results in an ubiquitin that is fully functional at the C-terminus, and with the full complement of reactive lysines to allow for poly-ubiquitin chain incorporation. This reagent allows for poly-ubiquitin chain incorporation of fluorescein-N-terminal ubiquitin with higher efficiency and detection sensitivity than traditionally modified ubiquitins.

Purity: ≥95% (SDS-PAGE) MW: ~8.6 kDa

Ubiquitin Fluorescein, human recombinant

U-590 100 µg

Ubiquitin modified with fluorescein via primary amine coupling. This results in multiple fluoresceinated ubiquitin species with modified lysines as well as the N-terminus. Although having a fully functional C-terminus, lysine modification may limit the ability of this reagent to efficiently incorporate into poly-ubiquitin chains.

Purity: ≥95% (SDS-PAGE) MW: ~8.6 kDa

Heavy Labeled Proteins

Ubiquitin-13C15N, human recombinant

U-700 100 µg

Isotopically labeled ubiquitin is useful in determining total cellular concentrations of ubiquitin, or determining the ratio of free- to substrate-bound ubiquitin using the protein standard absolute quantification (PSAQ) or related methods. Highly purified 13C/15N labeled ubiquitin is processed for the quantitative removal of glycine and buffer salts which can interfere with chemical and in vitro reactions. Ubiquitin is a 76 amino acid, highly conserved nuclear and cytoplasmic protein.

Purity: ≥95% (SDS-PAGE) MW: ~9.0 kDa

Methylated Ubiquitin-13C15N, human recombinant

U-720 100 µg

Isotopically labeled ubiquitin is useful in determining total cellular concentrations of ubiquitin, or determining the ratio of free- to substrate-bound ubiquitin using the protein standard absolute quantification (PSAQ) or related methods. Reductive methylation of lysine residues renders ubiquitin unable to form poly-ubiquitin chains via lysine linkages with other Ub molecules. Methylated Ub can form an E1-catalyzed active thioester at the C-terminus allowing the molecule to be transferred to the lysines of substrate proteins in a mono-ubiquitinated or multi-ubiquitinated fashion.

Purity: ≥95% (SDS-PAGE) MW: ~9.2 kDa

Rhodamine

Ubiquitin, N-Terminal Rhodamine, human recombinant

U-600 50 µg

Produced via a proprietary process resulting in a single rhodamine modification exclusively on the N-terminus of ubiquitin. This site-specific modification results in an ubiquitin that is fully functional at the C-terminus, and with the full complement of reactive lysines to allow for poly-ubiquitin chain incorporation. This reagent allows for poly-ubiquitin chain incorporation of rhodamine-N-terminal ubiquitin with higher efficiency and detection sensitivity than traditionally modified ubiquitins.

Purity: ≥95% (SDS-PAGE) MW: ~8.6 kDa
Ubiquitin Mutants

There are seven potential lysine residues (K6, K11, K27, K29, K33, K48 and K63) on ubiquitin that can participate in chain linkage formation. Thus, ubiquitin lysine mutants are useful reagents for in vitro studies designed to implicate given lysine residues in poly-ubiquitin chain initiation and conjugation events. Importantly, since the C-terminal residues are intact, these proteins are fully functional for activation and thioester formation by E1, E2 and E3 conjugating enzymes. These mutants can also be used in binding studies with proteins that contain ubiquitin recognition domains. These reagents are valuable for the determination of structure-function requirements in chain synthesis, recognition or disassembly.

Five ubiquitin lysine residues (K6, K11, K29, K48 and K63) are known to be sites of initiation and these linkages exist in vivo as anchored and unanchored species. The most abundant chain species are K48-linked and K63-linked poly-ubiquitins. The lysine (K) to arginine (R) mutants renders ubiquitin unable to form multi-ubiquitin chains via that specific lysine with other ubiquitin molecules. However, these proteins can still be linked to the lysine residues on target proteins formed via the remaining ubiquitin lysine residues that have not been mutated. The lysine (K) only mutants can only form poly-ubiquitin chains with other ubiquitin molecules via that single lysine, since all other lysines are absent. These mutants are ideal for the reduction in poly-ubiquitin chain length or conjugation rates and determining if the chains have a specific linkage requirement.

Ubiquitin chains adopt a structure where three amino acid residues (L8, I44 and V70) form a repeating hydrophobic patch that mediates intra-chain interactions, and thus is necessary and important for chain recognition by various factors. Mutation of I44 affects the structure of this hydrophobic surface and affects the recognition of ubiquitin chains by ubiquitin-binding domains such as ubiquitin interacting motifs (UIMs), ubiquitin associated domains (UBAs), coupling of ubiquitin to ER degradation (CUE), Nlp14 zinc finger (NZF), ubiquitin E2 variant (UEV) or proteasome subunits such as S5a.

### Lysine to Arginine

<table>
<thead>
<tr>
<th>Lysine</th>
<th>Mutant</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>K6</td>
<td>K6R</td>
<td>Mutation of lysine 6 to arginine renders ubiquitin (Ub) unable to form poly-Ub chains via lysine 6 linkages with other Ub molecules. Ub K6R can form an E1-catalyzed active thioester at the C-terminus allowing the molecule to be transferred to the lysines of substrate proteins. Ideal for the reduction in poly-Ub chain length/conjugation rates and for determining if poly-Ub chains are K6 linked.</td>
</tr>
<tr>
<td>K11</td>
<td>K11R</td>
<td>Mutation of lysine 11 to arginine renders ubiquitin (Ub) unable to form poly-Ub chains via lysine 11 linkages with other Ub molecules. Ub K11R can form an E1-catalyzed active thioester at the C-terminus allowing the molecule to be transferred to the lysines of substrate proteins. Ideal for the reduction in poly-Ub chain length/conjugation rates and for determining if poly-Ub chains are K11 linked.</td>
</tr>
</tbody>
</table>

**Ubiquitin Lysine Linkages**

<table>
<thead>
<tr>
<th>Lysine</th>
<th>Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys6</td>
<td>DNA Repair</td>
</tr>
<tr>
<td>Lys11</td>
<td>Unknown</td>
</tr>
<tr>
<td>Lys27</td>
<td>Proteasome</td>
</tr>
<tr>
<td>Lys29</td>
<td>Other Unknown Functions</td>
</tr>
<tr>
<td>Lys33</td>
<td>Proteasome</td>
</tr>
<tr>
<td>Lys48</td>
<td>IkB Activation</td>
</tr>
<tr>
<td>Lys63</td>
<td>Endocytosis, Mitotic Stress, Translation, DNA Repair</td>
</tr>
</tbody>
</table>

---

**Lysine to Arginine**

**Ubiquitin K6R, human recombinant**

<table>
<thead>
<tr>
<th>UM-K6R</th>
<th>1 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutations of lysine 6 to arginine renders ubiquitin (Ub) unable to form poly-Ub chains via lysine 6 linkages with other Ub molecules. Ub K6R can form an E1-catalyzed active thioester at the C-terminus allowing the molecule to be transferred to the lysines of substrate proteins. Ideal for the reduction in poly-Ub chain length/conjugation rates and for determining if poly-Ub chains are K6 linked.</td>
<td></td>
</tr>
<tr>
<td>Purity: ≥95% (SDS-PAGE)</td>
<td>MW: ~8.5 kDa</td>
</tr>
</tbody>
</table>

**His6-Ubiquitin K6R, human recombinant**

<table>
<thead>
<tr>
<th>UM-HK6R</th>
<th>1 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation of lysine 6 to arginine renders ubiquitin (Ub) unable to form poly-Ub chains via lysine 6 linkages with other Ub molecules. Ub K6R can form an E1-catalyzed active thioester at the C-terminus allowing the molecule to be transferred to the lysines of substrate proteins. Ideal for the reduction in poly-Ub chain length/conjugation rates and for determining if poly-Ub chains are K6 linked.</td>
<td></td>
</tr>
<tr>
<td>Purity: ≥95% (SDS-PAGE)</td>
<td>MW: ~9.3 kDa</td>
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</tbody>
</table>

**Ubiquitin K11R, human recombinant**

<table>
<thead>
<tr>
<th>UM-K11R</th>
<th>1 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation of lysine 11 to arginine renders ubiquitin (Ub) unable to form poly-Ub chains via lysine 11 linkages with other Ub molecules. Ub K11R can form an E1-catalyzed active thioester at the C-terminus allowing the molecule to be transferred to the lysines of substrate proteins. Ideal for the reduction in poly-Ub chain length/conjugation rates and for determining if poly-Ub chains are K11 linked.</td>
<td></td>
</tr>
<tr>
<td>Purity: ≥95% (SDS-PAGE)</td>
<td>MW: ~8.5 kDa</td>
</tr>
</tbody>
</table>

**His6-Ubiquitin K11R, human recombinant**

<table>
<thead>
<tr>
<th>UM-HK11R</th>
<th>1 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation of lysine 11 to arginine renders ubiquitin (Ub) unable to form poly-Ub chains via lysine 11 linkages with other Ub molecules. Ub K11R can form an E1-catalyzed active thioester at the C-terminus allowing the molecule to be transferred to the lysines of substrate proteins. Ideal for the reduction in poly-Ub chain length/conjugation rates and for determining if poly-Ub chains are K11 linked.</td>
<td></td>
</tr>
<tr>
<td>Purity: ≥95% (SDS-PAGE)</td>
<td>MW: ~9.3 kDa</td>
</tr>
<tr>
<td><strong>UBIQUITIN MUTANTS</strong></td>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
<td></td>
</tr>
</tbody>
</table>

**Ubiquitin K27R, human recombinant**

UM-K27R 1 mg

Mutation of lysine 27 to arginine renders ubiquitin (Ub) unable to form poly-Ub chains via lysine 27 linkages with other Ub molecules. Ub K27R can form an E1-catalyzed active thioester at the C-terminus allowing the molecule to be transferred to the lysines of substrate proteins. Ideal for the reduction in poly-Ub chain length/conjugation rates and determining if poly-Ub chains are K27 linked.

**Purity**: ≥95% (SDS-PAGE) **MW**: ~8.5 kDa

---

**His<sub>6</sub>-Ubiquitin K27R, human recombinant**

UM-HK27R 1 mg

Mutation of lysine 27 to arginine renders ubiquitin (Ub) unable to form poly-Ub chains via lysine 27 linkages with other Ub molecules. Ub K27R can form an E1-catalyzed active thioester at the C-terminus allowing the molecule to be transferred to the lysines of substrate proteins. Ideal for the reduction in poly-Ub chain length/conjugation rates and determining if poly-Ub chains are K27 linked.

**Purity**: ≥95% (SDS-PAGE) **MW**: ~9.3 kDa

---

**Ubiquitin K29R, human recombinant**

UM-K29R 1 mg

Mutation of lysine 29 to arginine renders ubiquitin (Ub) unable to form poly-Ub chains via lysine 29 linkages with other Ub molecules. Ub K29R can form an E1-catalyzed active thioester at the C-terminus allowing the molecule to be transferred to the lysines of substrate proteins. Ideal for the reduction in poly-Ub chain length/conjugation rates and determining if poly-Ub chains are K29 linked.

**Purity**: ≥95% (SDS-PAGE) **MW**: ~8.5 kDa

---

**His<sub>6</sub>-Ubiquitin K29R, human recombinant**

UM-HK29R 1 mg

Mutation of lysine 29 to arginine renders ubiquitin (Ub) unable to form poly-Ub chains via lysine 29 linkages with other Ub molecules. Ub K29R can form an E1-catalyzed active thioester at the C-terminus allowing the molecule to be transferred to the lysines of substrate proteins. Ideal for the reduction in poly-Ub chain length/conjugation rates and determining if poly-Ub chains are K29 linked.

**Purity**: ≥95% (SDS-PAGE) **MW**: ~9.3 kDa

---

**Ubiquitin K33R, human recombinant**

UM-K33R 1 mg

Mutation of lysine 33 to arginine renders ubiquitin (Ub) unable to form poly-Ub chains via lysine 33 linkages with other Ub molecules. Ub K33R can form an E1-catalyzed active thioester at the C-terminus allowing the molecule to be transferred to the lysines of substrate proteins. Ideal for the reduction in poly-Ub chain length/conjugation rates and determining if poly-Ub chains are K33 linked.

**Purity**: ≥95% (SDS-PAGE) **MW**: ~8.5 kDa

---

**His<sub>6</sub>-Ubiquitin K33R, human recombinant**

UM-HK33R 1 mg

Mutation of lysine 33 to arginine renders ubiquitin (Ub) unable to form poly-Ub chains via lysine 33 linkages with other Ub molecules. Ub K33R can form an E1-catalyzed active thioester at the C-terminus allowing the molecule to be transferred to the lysines of substrate proteins. Ideal for the reduction in poly-Ub chain length/conjugation rates and determining if poly-Ub chains are K33 linked.

**Purity**: ≥95% (SDS-PAGE) **MW**: ~9.3 kDa

---

**Ubiquitin K48R, human recombinant**

UM-K48R 1 mg

Mutation of lysine 48 to arginine renders ubiquitin (Ub) unable to form poly-Ub chains via lysine 48 linkages with other Ub molecules. Ub K48R can form an E1-catalyzed active thioester at the C-terminus allowing the molecule to be transferred to the lysines of substrate proteins (mono-ubiquitination). Ideal for the reduction in poly-Ub chain length/conjugation rates and for the determination of poly-Ub chains specificity.

**Purity**: ≥95% (SDS-PAGE) **MW**: ~8.5 kDa

---

**His<sub>6</sub>-Ubiquitin K48R, human recombinant**

UM-HK48R 1 mg

Mutation of lysine 48 to arginine renders ubiquitin (Ub) unable to form poly-Ub chains via lysine 48 linkages with other Ub molecules. Ub K48R can form an E1-catalyzed active thioester at the C-terminus allowing the molecule to be transferred to the lysines of substrate proteins (mono-ubiquitination). Ideal for the reduction in poly-Ub chain length/conjugation rates and determining if poly-Ub chains are K48 linked.

**Purity**: ≥95% (SDS-PAGE) **MW**: ~9.3 kDa

---

**Ubiquitin K63R, human recombinant**

UM-K63R 1 mg

Mutation of lysine 63 to arginine renders ubiquitin (Ub) unable to form poly-Ub chains via lysine 63 linkages with other Ub molecules. Ub K63R can form an E1-catalyzed active thioester at the C-terminus allowing the molecule to be transferred to the lysines of substrate proteins. Ideal for the reduction in poly-Ub chain length/conjugation rates and determining if poly-Ub chains are K63 linked.

**Purity**: ≥95% (SDS-PAGE) **MW**: ~8.5 kDa

---

**His<sub>6</sub>-Ubiquitin K63R, human recombinant**

UM-HK63R 1 mg

Mutation of lysine 63 to arginine renders ubiquitin (Ub) unable to form poly-Ub chains via lysine 63 linkages with other Ub molecules. Ub K63R can form an E1-catalyzed active thioester at the C-terminus allowing the molecule to be transferred to the lysines of substrate proteins. Ideal for the reduction in poly-Ub chain length/conjugation rates and determining if poly-Ub chains are K63 linked.

**Purity**: ≥95% (SDS-PAGE) **MW**: ~9.3 kDa

---

For research use only. Not for use in diagnostic procedures.
### UBIQUITIN MUTANTS

**Ubiquitin (K48R, K63R), human recombinant**

<table>
<thead>
<tr>
<th>Code</th>
<th>1 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>UM-K4863R</td>
<td></td>
</tr>
</tbody>
</table>

Mutation of lysine residues 48 and 63 to arginine renders ubiquitin unable to form poly-ubiquitin chains via these major known poly-ubiquitination sites. This ubiquitin double mutant can form an E1-catalyzed active thioester at the C-terminus allowing the molecule to be transferred to the lysines of substrate proteins.  

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~8.5 kDa

**Ubiquitin, (K29R, K48R, K63R), human recombinant**

<table>
<thead>
<tr>
<th>Code</th>
<th>1 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>UM-3KTR</td>
<td></td>
</tr>
</tbody>
</table>

Mutation of lysines 29, 48 and 63 to arginine renders ubiquitin unable to form poly-ubiquitin chains via the major known poly-ubiquitination sites. The ubiquitin triple mutant can form an E1-catalyzed active thioester at the C-terminus allowing the molecule to be transferred to the lysines of substrate proteins. Ideal for studies of alternate mono-ubiquitination or poly-ubiquitination sites.  

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~8.5 kDa

**Single Lysine**

**Ubiquitin K6 only, human recombinant**

<table>
<thead>
<tr>
<th>Code</th>
<th>1 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>UM-K60</td>
<td></td>
</tr>
</tbody>
</table>

This ubiquitin mutant contains only a single lysine, K6, with all other lysines mutated to arginine. This mutation renders ubiquitin able to form poly-ubiquitin chains with other ubiquitin molecules only via the K6 lysine.  

**Purity:** ≥95% by SDS-PAGE  
**MW:** ~8.5 kDa

**His<sub>6</sub>-Ubiquitin K6 only, human recombinant**

<table>
<thead>
<tr>
<th>Code</th>
<th>1 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>UM-HK60</td>
<td></td>
</tr>
</tbody>
</table>

This ubiquitin mutant contains only a single lysine, K6, with all other lysines mutated to arginine. This mutation renders ubiquitin able to form poly-ubiquitin chains with other ubiquitin molecules only via the K6 lysine.  

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~9.3 kDa

**Ubiquitin K11 only, human recombinant**

<table>
<thead>
<tr>
<th>Code</th>
<th>1 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>UM-K110</td>
<td></td>
</tr>
</tbody>
</table>

This ubiquitin mutant contains only a single lysine, K11, with all other lysines mutated to arginine. This mutation renders ubiquitin able to form poly-ubiquitin chains with other ubiquitin molecules only via the K11 lysine.  

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~8.5 kDa

**His<sub>6</sub>-Ubiquitin K11 only, human recombinant**

<table>
<thead>
<tr>
<th>Code</th>
<th>1 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>UM-HK110</td>
<td></td>
</tr>
</tbody>
</table>

This ubiquitin mutant contains only a single lysine, K11, with all other lysines mutated to arginine. This mutation renders ubiquitin able to form poly-ubiquitin chains with other ubiquitin molecules only via the K11 lysine.  

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~9.3 kDa

**Ubiquitin K27 only, human recombinant**

<table>
<thead>
<tr>
<th>Code</th>
<th>1 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>UM-K270</td>
<td></td>
</tr>
</tbody>
</table>

This ubiquitin mutant contains only a single lysine, K27, with all other lysines mutated to arginine. This mutation renders ubiquitin able to form poly-ubiquitin chains with other ubiquitin molecules only via the K27 lysine.  

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~8.5 kDa

**His<sub>6</sub>-Ubiquitin K27 only, human recombinant**

<table>
<thead>
<tr>
<th>Code</th>
<th>1 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>UM-HK270</td>
<td></td>
</tr>
</tbody>
</table>

This ubiquitin mutant contains only a single lysine, K27, with all other lysines mutated to arginine. This mutation renders ubiquitin able to form poly-ubiquitin chains with other ubiquitin molecules only via the K27 lysine.  

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~9.3 kDa

**Ubiquitin K29 only, human recombinant**

<table>
<thead>
<tr>
<th>Code</th>
<th>1 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>UM-K290</td>
<td></td>
</tr>
</tbody>
</table>

This ubiquitin mutant contains only a single lysine, K29, with all other lysines mutated to arginine. This mutation renders ubiquitin able to form poly-ubiquitin chains with other ubiquitin molecules only via the K29 lysine.  

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~8.5 kDa

**His<sub>6</sub>-Ubiquitin K29 only, human recombinant**

<table>
<thead>
<tr>
<th>Code</th>
<th>1 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>UM-HK290</td>
<td></td>
</tr>
</tbody>
</table>

This ubiquitin mutant contains only a single lysine, K29, with all other lysines mutated to arginine. This mutation renders ubiquitin able to form poly-ubiquitin chains with other ubiquitin molecules only via the K29 lysine.  

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~9.3 kDa

**Ubiquitin K33 only, human recombinant**

<table>
<thead>
<tr>
<th>Code</th>
<th>1 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>UM-K330</td>
<td></td>
</tr>
</tbody>
</table>

This ubiquitin mutant contains only a single lysine, K33, with all other lysines mutated to arginine. This mutation renders ubiquitin able to form poly-ubiquitin chains with other ubiquitin molecules only via the K33 lysine.  

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~8.5 kDa

**His<sub>6</sub>-Ubiquitin K33 only, human recombinant**

<table>
<thead>
<tr>
<th>Code</th>
<th>1 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>UM-HK330</td>
<td></td>
</tr>
</tbody>
</table>

This ubiquitin mutant contains only a single lysine, K33, with all other lysines mutated to arginine. This mutation renders ubiquitin able to form poly-ubiquitin chains with other ubiquitin molecules only via the K33 lysine.  

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~9.3 kDa

**Ubiquitin K48 only, human recombinant**

<table>
<thead>
<tr>
<th>Code</th>
<th>1 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>UM-K480</td>
<td></td>
</tr>
</tbody>
</table>

This ubiquitin mutant contains only a single lysine, K48, with all other lysines mutated to arginine. This mutation renders ubiquitin able to form poly-ubiquitin chains with other ubiquitin molecules exclusively via the K48 lysine.  

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~8.5 kDa
### UBIQUITIN MUTANTS

**His<sub>6</sub>-Ubiquitin K48 only, human recombinant**

UM-HK480 1 mg

This ubiquitin mutant contains only a single lysine, K48, with all other lysines mutated to arginine. This mutation renders ubiquitin able to form poly-ubiquitin chains with other ubiquitin molecules only via the K48 lysine.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~9.3 kDa

**Ubiquitin K63 only, human recombinant**

UM-K630 1 mg

This ubiquitin mutant contains only a single lysine, K63, with all other lysines mutated to arginine. This mutation renders ubiquitin able to form poly-ubiquitin chains with other ubiquitin molecules only via the K63 lysine.

**Purity:** >95% (SDS-PAGE)  **MW:** ~8.5 kDa

**His<sub>6</sub>-Ubiquitin K63 only, human recombinant**

UM-HK630 1 mg

This ubiquitin mutant contains only a single lysine, K63, with all other lysines mutated to arginine. This mutation renders ubiquitin able to form poly-ubiquitin chains with other ubiquitin molecules only via the K63 lysine.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~9.3 kDa

**Ubiquitin, No Lysines, human recombinant**

UM-NOK 1 mg

This ubiquitin mutant contains no lysine residues, since all lysines are mutated to arginine. This mutation renders ubiquitin unable to form poly-ubiquitin chains and is useful as a negative control.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~8.5 kDa

**His<sub>6</sub>-Ubiquitin, No Lysines, human recombinant**

UM-HNOK 1 mg

This ubiquitin mutant contains no lysine residues, since all lysines are mutated to arginine. This mutation renders ubiquitin unable to form poly-ubiquitin chains and is useful as a negative control.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~9.3 kDa

### Other Mutants

**Ubiquitin F4A, human recombinant**

UM-F4A 1 mg

Ubiquitin has two functional surfaces which are critical for various signaling processes. The hydrophobic patch (Leu<sup>8</sup>, Ile<sup>44</sup> and Val<sup>70</sup>) is important for binding to the proteasome, UIM (ubiquitin interacting motif) and UBA (ubiquitin associated) domains. This multifunctional surface thus influences many ubiquitination and deubiquitination reactions. The other surface defined by Phe<sup>4</sup> is required specifically for non-proteasome-dependent functions such as endocytosis and internalization, which often involves mono-ubiquitination. It is thought that Phe<sup>4</sup> may be involved in specific protein-protein interactions that facilitate endocytosis. In addition, Ile<sup>44</sup> forms a di-leucine signal with Leu<sup>43</sup> that may be involved in mediating endocytosis of substrate proteins that are mono-ubiquitinated. Ubiquitin F4A can form an E1-catalyzed active thioester at the C-terminus allowing the molecule to be transferred to the lysines of substrate proteins.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~8.5 kDa

**Ubiquitin L8A, human recombinant**

UM-L8A 1 mg

Ubiquitin has a distinct and functionally important hydrophobic patch whose surface is defined by three residues including Leu<sup>8</sup>, Ile<sup>44</sup> and Val<sup>70</sup>. These residues are solvent accessible in ubiquitin chains and are critical for poly-ubiquitin chain interaction and recognition by the 26S proteasome and subsequent target degradation. These hydrophobic residues interact directly with various factors that bind to Ub and/or mediate Ub-Ub interactions in ubiquitin chains that may influence orientation and recognition. Ubiquitin L8A can form an E1-catalyzed active thioester at the C-terminus allowing the molecule to be transferred to the lysines of substrate proteins.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~8.5 kDa

**Ubiquitin I44A, human recombinant**

UM-I44A 1 mg

Isoleucine 44 is a surface hydrophobic residue that is important for poly-ubiquitin chain interaction and recognition by the 26S proteasome and other enzymes involved in ubiquitination. Ub I44A can form an E1-catalyzed active thioester at the C-terminus allowing the molecule to be transferred to the lysines of substrate proteins.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~8.5 kDa

**Ubiquitin D58A, human recombinant**

UM-D58A 1 mg

D58 is a residue that has been identified as being important for binding and recognition by proteins that contain ubiquitin binding domains (UBDs), and represents a new hydrophilic interaction surface on ubiquitin. This residue may be crucial for the interaction and recognition of poly-ubiquitin chains by the 26S proteasome and other enzymes involved in ubiquitination. Ub D58A can form an E1-catalyzed active thioester at the C-terminus allowing the molecule to be transferred to the lysines of substrate proteins.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~8.5 kDa

For research use only. Not for use in diagnostic procedures.
### C-Terminal Mutants

#### **Ubiquitin V70A, human recombinant**

<table>
<thead>
<tr>
<th>UM-V70A</th>
<th>1 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ubiquitin has a distinct and functionally important hydrophobic patch whose surface is defined by three residues including Leu9, Ile44 and Val70. These residues are solvent accessible in ubiquitin chains and are critical for poly-ubiquitin chain interaction and recognition by the 26S proteasome and subsequent target degradation. These hydrophobic residues interact directly with various factors that bind to Ub and/or mediate Ub-Ub interactions in ubiquitin chains that may influence orientation and recognition. Ubiquitin V70A can form an E1-catalyzed active thioester at the C-terminus allowing the molecule to be transferred to the lysines of substrate proteins.</td>
<td></td>
</tr>
<tr>
<td><strong>Purity:</strong> ≥95% (SDS-PAGE) <strong>MW:</strong> ~8.5 kDa</td>
<td></td>
</tr>
</tbody>
</table>

#### **His<sub>6</sub>-Ubiquitin-AA, human recombinant**

<table>
<thead>
<tr>
<th>UM-HAA</th>
<th>100 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mature forms of Ub have a highly conserved diglycine motif at the carboxyl terminus which is crucial for activity and recognition in conjugation and deconjugation reactions. The replacement of this diglycine peptide with two alanine residues results in an inactive Ub. This Ub cannot be activated by the E1 enzyme in an ATP-dependent manner, is not capable of subsequent thioester interaction with E2 and/or E3 enzymes, and is thus not capable of forming isopeptide bonds or Ub conjugates. It can be used as a negative control in conjugation reactions, or in binding studies with E1, E2, E3 and DUB enzymes or other proteins that interact with ubiquitin via ubiquitin-associated domains (UBAs) or ubiquitin-interacting motifs (UIMs). This protein contains an N-terminal His&lt;sub&gt;6&lt;/sub&gt;-tag.</td>
<td></td>
</tr>
<tr>
<td><strong>Purity:</strong> ≥95% (SDS-PAGE) <strong>MW:</strong> ~9.3 kDa</td>
<td></td>
</tr>
</tbody>
</table>

#### **Ubiquitin-R74, human recombinant**

<table>
<thead>
<tr>
<th>UM-R74</th>
<th>100 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mature forms of ubiquitin have a highly conserved diglycine motif at the carboxyl terminus which is crucial for activity and recognition in conjugation and deconjugation reactions. The removal of this diglycine peptide (Ub ending in Arg74) results in an inactive Ub. This Ub cannot be activated by the E1 enzyme in an ATP-dependent manner, is not capable of subsequent thioester interaction with E2 and/or E3 enzymes, and is thus not capable of forming isopeptide bonds or Ub conjugates. It can be used as a negative control in conjugation reactions, or in binding studies with E1, E2, E3 and DUB enzymes or other proteins that interact with ubiquitin via ubiquitin-associated domains (UBAs) or ubiquitin-interacting motifs (UIMs).</td>
<td></td>
</tr>
<tr>
<td><strong>Purity:</strong> ≥95% (SDS-PAGE) <strong>MW:</strong> ~8.5 kDa</td>
<td></td>
</tr>
</tbody>
</table>

#### **His<sub>6</sub>-Ubiquitin-R74, human recombinant**

<table>
<thead>
<tr>
<th>UM-HR74</th>
<th>100 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mature forms of ubiquitin have a highly conserved diglycine motif at the carboxyl terminus which is crucial for activity and recognition in conjugation and deconjugation reactions. The removal of this diglycine peptide (Ub ending in Arg74) results in an inactive Ub. This Ub cannot be activated by the E1 enzyme in an ATP-dependent manner, is not capable of subsequent thioester interaction with E2 and/or E3 enzymes, and is thus not capable of forming isopeptide bonds or Ub conjugates. It can be used as a negative control in conjugation reactions, or in binding studies with E1, E2, E3 and DUB enzymes or other proteins that interact with ubiquitin via ubiquitin-associated domains (UBAs) or ubiquitin-interacting motifs (UIMs).</td>
<td></td>
</tr>
<tr>
<td><strong>Purity:</strong> ≥95% (SDS-PAGE) <strong>MW:</strong> ~9.3 kDa</td>
<td></td>
</tr>
</tbody>
</table>

#### **Ubiquitin-G76A, human recombinant**

<table>
<thead>
<tr>
<th>UM-G76A</th>
<th>100 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mature Ub has a highly conserved C-terminal diglycine motif which is crucial for activity and recognition with conjugation and deconjugation enzyme components. The replacement of the last glycine residue with alanine results in a Ub that supports E1-Ub thioester formation and downstream conjugation reactions (transfer to E2, E3) but at a rate ~20% compared to wildtype Ub. This mutant however, inhibits deconjugation and prevents the removal of Ub from modified protein substrates by deubiquitinating enzyme (DUBs). Since this Ub becomes irreversibly conjugated to protein, it shifts the equilibrium between the bound and unbound form in the direction of conjugation, at the expense of the free form.</td>
<td></td>
</tr>
<tr>
<td><strong>Purity:</strong> ≥95% (SDS-PAGE) <strong>MW:</strong> ~8.5 kDa</td>
<td></td>
</tr>
</tbody>
</table>

#### **His<sub>6</sub>-Ubiquitin-G76A, human recombinant**

<table>
<thead>
<tr>
<th>UM-HG76A</th>
<th>100 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mature Ub has a highly conserved C-terminal diglycine motif which is crucial for activity and recognition with conjugation and deconjugation enzyme components. The replacement of the last glycine residue with alanine results in a Ub that supports E1-Ub thioester formation and downstream conjugation reactions (transfer to E2, E3) but at a rate ~20% compared to wildtype Ub. This mutant however, inhibits deconjugation and prevents the removal of Ub from modified protein substrates by deubiquitinating enzyme (DUBs). Since this Ub becomes irreversibly conjugated to protein, it shifts the equilibrium between the bound and unbound form in the direction of conjugation, at the expense of the free form.</td>
<td></td>
</tr>
<tr>
<td><strong>Purity:</strong> ≥95% (SDS-PAGE) <strong>MW:</strong> ~8.5 kDa</td>
<td></td>
</tr>
</tbody>
</table>
Ubiquitin Chains

The type and number of poly-ubiquitin chains that are conjugated to a target are highly regulated to generate distinct signals that affect different physiological processes. This versatility arises from the fact that not only can targets be mono-ubiquitinated or poly-ubiquitinated, but also that different types of poly-ubiquitin chains are formed. Proteins tagged with ubiquitin are most often destined for degradation by the proteasome. However, mono-ubiquitination and poly-ubiquitination also have non-proteasomal regulatory functions like targeting proteins to nucleus, cytoskeleton and endocytic machinery, or modulating enzymatic activity and protein-protein interactions.

Multi-ubiquitin chains are built by formation of an isopeptide bond between Gly76 of one ubiquitin to the a-NH2 group of one of the seven potential lysines (K6, K11, K27, K29, K33, K48 or K63) of the preceding ubiquitin. Specific E2 enzymes and E2/E3 combinations result in the formation of linkage-specific ubiquitin chains. Residue K48 is a major site of chain initiation and K48 linkages are highly abundant, being the predominant signal for proteins destined for degradation by the proteasome. The other principal and relatively abundant poly-ubiquitin chain has K63 linkages. K63 linkages do not seem to play a role in protein turnover and have been implicated in receptor endocytosis and sorting, translation, DNA damage repair, the stress response and signaling through the TRAF pathway of NF-kB. Since K48- and K63-linked Ub chains have such different targeting functions, they may have unique chain structures which result in selective recognition by regulatory components. Ubiquitin polymers with G76-K6, G76-K11 and G76-K29 linkages are minor species in vivo and there is currently no evidence that K27 and K33 are used to form ubiquitin-ubiquitin isopeptide linkages.

K6-linked Chains

Di-Ub WT Chains (Ub2)

UC-11 25 µg

Linkage specific di-Ub is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. It can also be used to investigate mechanism of binding and recognition by E1 or E2 enzymes, deubiquitinating enzymes, E3 ligases or other proteins that contain ubiquitin-associated domains (UBAs) or ubiquitin-interacting motifs (UIMs).

Purity: ≥90% (SDS-PAGE) MW: ~17 kDa

K11-linked Chains

Di-Ub WT Chains (Ub2), human recombinant

UC-40 25 µg

Linkage specific di-Ub is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. It can also be used to investigate mechanism of binding and recognition by E1 or E2 enzymes, DUBs, E3 ligases or other proteins that contain ubiquitin-associated domains (UBAs) or ubiquitin-interacting motifs (UIMs). This product is formed with wild-type human recombinant ubiquitin and linkage-specific enzymes. Whereas K48-linked Ub chains mediate proteasomal degradation, and K63-linked Ub chains act in non-proteolytic events, the roles of unconventional poly-ubiquitin chains (linked through K6, K11, K27, K29, or K33) are not yet well understood. These non-canonical linkages are abundant in vivo and they may also target proteins for degradation. Ubiquitination through K11 is also important for ER-associated degradation (ERAD) and there undoubtedly exist DUBs that may have specificity for K11 linkages.

Purity: ≥90% (SDS-PAGE) MW: ~17 kDa

K27-linked Chains

Di-Ub WT Chains (Ub2)

UC-61 25 µg

Linkage specific di-Ub is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. It can also be used to investigate mechanism of binding and recognition by E1 or E2 enzymes, deubiquitinating enzymes, E3 ligases or other proteins that contain ubiquitin-associated domains (UBAs) or ubiquitin-interacting motifs (UIMs).

Purity: ≥90% (SDS-PAGE) MW: ~17 kDa
### K29-linked Chains

**Di-Ub WT Chains (Ub$_2^\text{K29}$.)**

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Weight</th>
<th>Purity</th>
<th>MW</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>UC-81</td>
<td>25 µg</td>
<td>≥90% (SDS-PAGE)</td>
<td>~17 kDa</td>
<td>Linkage specific di-Ub is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. It can also be used to investigate the mechanism of binding and recognition by E1 or E2 enzymes, deubiquitinating enzymes, E3 ligases or other proteins that contain ubiquitin-associated domains (UBAs) or ubiquitin-interacting motifs (UIMs). Purity: ≥90% (SDS-PAGE) MW: ~17 kDa</td>
</tr>
</tbody>
</table>

### K33-linked Chains

**Di-Ub WT Chains (Ub$_2^\text{K33}$.)**

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Weight</th>
<th>Purity</th>
<th>MW</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>UC-101</td>
<td>25 µg</td>
<td>≥90% (SDS-PAGE)</td>
<td>~17 kDa</td>
<td>Linkage specific di-Ub is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. It can also be used to investigate the mechanism of binding and recognition by E1 or E2 enzymes, deubiquitinating enzymes, E3 ligases or other proteins that contain ubiquitin-associated domains (UBAs) or ubiquitin-interacting motifs (UIMs). Purity: ≥90% (SDS-PAGE) MW: ~17 kDa</td>
</tr>
</tbody>
</table>

### K48-linked Chains

**Di-Ub WT Chains (Ub$_2^\text{K48}$.), human recombinant**

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Weight</th>
<th>Purity</th>
<th>MW</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>UC-200</td>
<td>100 µg</td>
<td>≥90% (SDS-PAGE)</td>
<td>~17 kDa</td>
<td>Linkage specific di-Ub is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. It can also be used to investigate the mechanism of binding and recognition by E1 or E2 enzymes, deubiquitinating enzymes, E3 ligases or other proteins that contain ubiquitin-associated domains (UBAs) or ubiquitin-interacting motifs (UIMs). This product is formed with wild-type human recombinant ubiquitin and linkage-specific enzymes. Purity: ≥90% (SDS-PAGE) MW: ~17 kDa</td>
</tr>
</tbody>
</table>

**Biotin Tetra-Ub WT Chains (Ub$_4^\text{K48}$.)**

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Weight</th>
<th>Purity</th>
<th>MW</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCB-210</td>
<td>25 µg</td>
<td>&gt;90% (SDS-PAGE)</td>
<td>~34 kDa</td>
<td>Linkage-specific Tetra-Ub can also be used to investigate the mechanism of binding and recognition by E1 or E2 enzymes, deubiquitinating enzymes, E3 ligases or other proteins that contain ubiquitin-associated domains (UBAs) or ubiquitin-interacting motifs (UIMs). This product is formed with wild-type human recombinant ubiquitin and linkage-specific enzymes. Purity: &gt;90% (SDS-PAGE) MW: ~34 kDa</td>
</tr>
</tbody>
</table>
**Poly-Ub WT Chains (Ub\textsubscript{3-7}), human recombinant**

UC-220 100 µg

Linkage specific poly-ubiquitin chains are used to investigate mechanisms of chain recognition, binding and hydrolysis by the proteasome, deubiquitinating enzymes, E3 ligases or other proteins that contain ubiquitin-associated domains (UBAs) or ubiquitin-interacting motifs (UIMs). Lys48-linked chains are abundant \textit{in vivo} and act as a universal signal for proteasomal degradation. This product is formed with wild-type human recombinant ubiquitin and linkage-specific enzymes. This mixture of poly-ubiquitin chains contains tri-ubiquitin and higher MW species; mono- and di-ubiquitin have been removed.

**Purity:** ≥95% (SDS-PAGE)

**Biotin Poly-Ub WT Chains (Ub\textsubscript{3-7})**

UCB-230 50 µg

Linkage-specific poly-ubiquitin chains are used to investigate mechanisms of chain recognition, binding and hydrolysis by the proteasome, deubiquitinating enzymes, E3 ligases or other proteins that contain ubiquitin-associated domains or ubiquitin-interacting motifs (UIMs). Lys48-linked chains are abundant \textit{in vivo} and act as a universal signal for proteasomal degradation. This product is formed with wild-type human recombinant ubiquitin and linkage-specific enzymes. This mixture of poly-ubiquitin chains contains di-ubiquitin and higher MW species; mono-ubiquitin has been removed. These chains have been modified with biotin via primary amine coupling. This results in multiple biotinylated species modified at the N-terminus, as well as lysine residues. Biotinylated ubiquitin can be detected using avidin-linked reagents.

**Purity:** >90% (SDS-PAGE) **MW:** ~9.0 kDa

**Poly-Ub WT Chains (Ub\textsubscript{1-7}), human recombinant**

UC-240 100 µg

Linkage specific poly-ubiquitin chains are used to investigate mechanisms of chain recognition, binding and hydrolysis by the proteasome, deubiquitinating enzymes, E3 ligases or other proteins that contain ubiquitin-associated domains or ubiquitin-interacting motifs (UIMs). Lys48-linked chains are abundant \textit{in vivo} and act as a universal signal for proteasomal degradation. This product is formed with wild-type human recombinant ubiquitin and linkage-specific enzymes. This mixture of poly-ubiquitin chains contains tri-ubiquitin and higher MW species; mono-ubiquitin has been removed.

**Purity:** ≥95% (SDS-PAGE)

**His\textsubscript{6}-Poly-Ub WT Chains (Ub\textsubscript{1-7}), human recombinant**

UCH-230 100 µg

Linkage specific poly-Ub chains are used to investigate mechanisms of chain recognition, binding and hydrolysis by the proteasome, deubiquitinating enzymes, E3 ligases or other proteins that contain Ub-associated domains (UBAs) or ubiquitin-interacting motifs (UIMs). Lys48-linked chains are abundant \textit{in vivo} and act as a universal signal for proteasomal degradation. This product is formed with His\textsubscript{6}-tagged wild-type human recombinant Ub and linkage-specific enzymes. This mixture of poly-Ub chains contains di-Ub and higher MW species with mono-ubiquitin has been removed. The His\textsubscript{6}-tag is convenient for affinity purification and immuno-detection.

**Purity:** ≥95% (SDS-PAGE)
Tetra-Ub WT Chains (Ub-K48-Ub-K63-Ub-K48-Ub), human recombinant

UCM-310 25 µg

Tetra-Ub can be used to investigate mechanisms of binding and recognition by deubiquitinating enzymes, E3 ligases or other proteins that contain ubiquitin-associated domains (UBAs) or ubiquitin-interacting motifs (UIMs). Tetra-Ub is the minimal unit necessary for recognition by the 26S proteasome and contains structural characteristics (such as repeating hydrophobic patches) not present in di-Ub. This product is made with wild-type human recombinant ubiquitin and linkage-specific enzymes, which results in one K63-linkage between two K48-linkages.

Purity: ≥95% (SDS-PAGE) MW: ~34 kDa

Tri-Ub WT Chains (Ub3), human recombinant

UC-315 100 µg

Linkage specific tri-Ub can be used as a substrate for enzymes that cleave the isopeptide linkage between ubiquitin molecules. It can also be used to investigate mechanisms of binding and recognition by E1 or E2 enzymes, deubiquitinating enzymes, E3 ligases or other proteins that contain ubiquitin-associated domains (UBAs) or ubiquitin-interacting motifs (UIMs). This product is formed with wild-type human recombinant ubiquitin and linkage-specific enzymes.

Purity: ≥95% (SDS-PAGE) MW: ~26 kDa

Penta-Ub WT Chains (Ub5), human recombinant

UC-316 25 µg

Linkage specific penta-Ub can be used to investigate mechanisms of binding and recognition by E1 or E2 enzymes, deubiquitinating enzymes, E3 ligases, the proteasome or other proteins that contain ubiquitin-associated domains (UBAs) or ubiquitin interacting motifs (UIMs). This product is formed with wild-type human recombinant ubiquitin and linkage-specific enzymes. It has been shown that the rate of ubiquitin-substrate conjugate degradation is related to poly-ubiquitin chain length. Tetra-ubiquitin is the minimal unit required for recognition by the proteasome, and longer chains probably have enhanced binding to proteasomal subunits and may be more resistant to disassembly by proteasome-associated isopeptidases.

Purity: ≥90% (SDS-PAGE) MW: ~43 kDa

Hexa-Ub WT Chains (Ub6), human recombinant

UC-317 25 µg

Linkage specific hexa-Ub can be used to investigate mechanisms of binding and recognition by E1 or E2 enzymes, deubiquitinating enzymes, E3 ligases, the proteasome or other proteins that contain ubiquitin-associated domains (UBAs) or ubiquitin interacting motifs (UIMs). This product is formed with wild-type ubiquitin and linkage-specific enzymes. It has been shown that the rate of ubiquitin-substrate conjugate degradation is related to poly-ubiquitin chain length. Tetra-ubiquitin is the minimal unit required for recognition by the proteasome, and longer chains probably have enhanced binding to proteasomal subunits and may be more resistant to disassembly by proteasome-associated isopeptidases.

Purity: ≥90% (SDS-PAGE) MW: ~52 kDa

Octa-Ub WT Chains (Ub8), human recombinant

UC-318 25 µg

Linkage specific octa-Ub can be used to investigate mechanisms of binding and recognition by E1 or E2 enzymes, deubiquitinating enzymes, E3 ligases, the proteasome or other proteins that contain ubiquitin-associated domains (UBAs) or ubiquitin-interacting motifs (UIMs). This product is formed with wild-type ubiquitin and linkage-specific enzymes. It has been shown that the rate of ubiquitin-substrate conjugate degradation is related to poly-ubiquitin chain length. Tetra-ubiquitin is the minimal unit required for recognition by the proteasome, and longer chains probably have enhanced binding to proteasomal subunits and may be more resistant to disassembly by proteasome-associated isopeptidases.

Purity: ≥90% (SDS-PAGE) MW: ~69 kDa

Poly-Ub WT Chains (Ub3-7), human recombinant

UC-320 100 µg

Linkage specific poly-ubiquitin chains are used to investigate mechanisms of chain recognition, binding and hydrolysis by the proteasome, deubiquitinating enzymes, E3 ligases or other proteins that contain ubiquitin-associated domains (UBAs) or ubiquitin-interacting motifs (UIMs). Lys63-linked poly-ubiquitin has been implicated in several non-degradative processes such as receptor endocytosis and sorting, translation, DNA damage repair, the stress response and signaling in the NFκB pathway. This product is formed with wild-type ubiquitin and linkage-specific enzymes. This mixture of poly-ubiquitin chains contains tri-ubiquitin and higher MW species; mono- and di-ubiquitin have been removed.

Purity: ≥95% (SDS-PAGE)
Biotin Poly-Ub WT Chains (Ub_{1-7})

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCB-330</td>
<td>50 µg</td>
</tr>
</tbody>
</table>

Linkage-specific poly-ubiquitin chains are used to investigate mechanisms of chain recognition, binding and hydrolysis by the proteasome, deubiquitinating enzymes, E3 ligases or other proteins that contain ubiquitin-associated domains or ubiquitin-interacting motifs (UIMs). This product is formed with wild-type human recombinant ubiquitin and linkage-specific enzymes. This mixture of poly-ubiquitin chains contains di-ubiquitin and higher MW species; mono-ubiquitin has been removed. These chains have been modified with biotin via primary amine coupling. This results in multiple biotinylated species modified at the N-terminus, as well as lysine residues. Biotinylated ubiquitin can be detected using avidin-linked reagents.

**Purity:** ≥90% (SDS-PAGE) MW: ~9.0 kDa

Poly-Ub WT Chains (Ub_{1-7}), human recombinant

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>UC-330</td>
<td>100 µg</td>
</tr>
</tbody>
</table>

Linkage-specific poly-ubiquitin chains are used to investigate mechanisms of chain recognition, binding and hydrolysis by the proteasome, deubiquitinating enzymes, E3 ligases or other proteins that contain ubiquitin-associated domains (UBAs) or ubiquitin-interacting motifs (UIMs). Lys63-linked poly-ubiquitin has been implicated in several non-degradative processes such as receptor endocytosis and sorting, translation, DNA damage repair, the stress response and signaling in the NFκB pathway.

This product is formed with wild-type human recombinant ubiquitin and linkage-specific enzymes. This mixture of poly-ubiquitin chains contains di-ubiquitin and higher MW species; mono-ubiquitin has been removed. **Purity:** ≥95% (SDS-PAGE)

### Other Chains

**Linear Di-Ubiquitin (Ub_2), human recombinant**

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>UC-700</td>
<td>100 µg</td>
</tr>
</tbody>
</table>

This linear ubiquitin fusion protein can be used as a substrate for deubiquitinating enzymes (DUBs) to test for non-isopeptide bond cleavage activity or preference. Ub is not expressed directly as free Ub but rather as linear fusions either to itself or to certain ribosomal protein subunits. These Ub-fusion precursors are proteolyzed by (DUBs) at the appropriate junction points to yield active Ub monomers with C-termi ending in GG. There are likely several intracellular DUBs which perform this essential processing role. This protein can also be used to investigate mechanism of binding and recognition by E1 or E2 enzymes, DUBs, E3 ligases or other proteins that contain ubiquitin-associated domains (UBAs) or ubiquitin-interacting motifs (UIMs).

**Purity:** ≥95% (SDS-PAGE) MW: ~17 kDa

**Linear Tetra-Ubiquitin (Ub_4), human recombinant**

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>UC-710</td>
<td>100 µg</td>
</tr>
</tbody>
</table>

This linear ubiquitin fusion protein can be used as a substrate for deubiquitinating enzymes (DUBs) to test for non-isopeptide bond cleavage activity or preference. Ub is not expressed directly as free Ub but rather as linear fusions either to itself or to certain ribosomal protein subunits. These Ub-fusion precursors are proteolyzed by (DUBs) at the appropriate junction points to yield active Ub monomers with C-termi ending in GG. There are likely several intracellular DUBs which perform this essential processing role. This protein can also be used to investigate mechanism of binding and recognition by E1 or E2 enzymes, DUBs, E3 ligases or other proteins that contain ubiquitin-associated domains (UBAs) or ubiquitin-interacting motifs (UIMs).

**Purity:** ≥95% (SDS-PAGE) MW: ~35 kDa
Some signaling pathways may also be useful in exploring the role of unanchored poly-ubiquitin chains (such as repeating hydrophobic patches) not present in di-ubiquitin. This product may also be useful in exploring the role of unanchored poly-ubiquitin chains in some signaling pathways.

Purity: ≥95% (SDS-PAGE)  MW: ~17 kDa

**Di-Ub/Ub$_2$ (K48-linked), Agarose, human recombinant**

**UCN-200**  100 µL  
Linkage specific, non-hydrolyzable di-ubiquitin is resistant to the activity of enzymes (DUBs) that cleave the isopeptide linkage between adjacent ubiquitin molecules. It can be used to investigate binding interactions between di-ubiquitin and proteins that contain elements such as ubiquitin-associated domains (UBAs) or ubiquitin-interacting motifs (UIMs). This product may also be useful in exploring the role of unanchored poly-ubiquitin chains in some signaling pathways.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~17 kDa

**Tetra-Ub/Ub$_4$ (K48-linked), human recombinant**

**UCN-210**  25 µg  
Linkage specific, non-hydrolyzable tetra-ubiquitin is resistant to the activity of enzymes (DUBs) that cleave the isopeptide linkage between adjacent ubiquitin molecules. It can be used to investigate binding interactions between di-ubiquitin and proteins that contain elements such as ubiquitin-associated domains (UBAs) or ubiquitin-interacting motifs (UIMs). Tetra-ubiquitin is the minimal unit necessary for recognition by the 26S proteasome and contains structural characteristics (such as repeating hydrophobic patches) not present in di-ubiquitin. This product may also be useful in exploring the role of unanchored poly-ubiquitin chains in some signaling pathways.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~34 kDa

**Di-Ub/Ub$_2$ (K48-linked), human recombinant**

**UCN-202**  250 µL  
Linkage specific, non-hydrolyzable di-ubiquitin is resistant to the activity of enzymes (DUBs) that cleave the isopeptide linkage between adjacent ubiquitin molecules. It can be used to investigate binding interactions between di-ubiquitin and proteins that contain elements such as ubiquitin-associated domains (UBAs) or ubiquitin-interacting motifs (UIMs). This product may also be useful in exploring the role of unanchored poly-ubiquitin chains in some signaling pathways.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~17 kDa

**Tetra-Ub/Ub$_4$ (K48-linked), human recombinant**

**UCN-211**  100 µL  
Linkage specific, non-hydrolyzable tetra-ubiquitin is resistant to the activity of enzymes (DUBs) that cleave the isopeptide linkage between adjacent ubiquitin molecules. It can be used to investigate binding interactions between tri-ubiquitin and proteins that contain elements such as ubiquitin-associated domains (UBAs) or ubiquitin-interacting motifs (UIMs). Tetra-ubiquitin is the minimal unit necessary for recognition by the 26S proteasome and contains structural characteristics (such as repeating hydrophobic patches) not present in di-ubiquitin. This product may also be useful in exploring the role of unanchored poly-ubiquitin chains in some signaling pathways.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~26 kDa

**Di-Ub/Ub$_2$ (K48-linked), Agarose, human recombinant**

**UCN-200**  100 µg  
Linkage specific, non-hydrolyzable di-ubiquitin is resistant to the activity of enzymes (DUBs) that cleave the isopeptide linkage between adjacent ubiquitin molecules. It can be used to investigate binding interactions between tri-ubiquitin and proteins that contain elements such as ubiquitin-associated domains (UBAs) or ubiquitin-interacting motifs (UIMs). This product may also be useful in exploring the role of unanchored poly-ubiquitin chains in some signaling pathways.
UBIQUITIN CHAINS

**Tetra-Ub/Ub₄ (K48-linked), human recombinant**

| UCN-312 | 100 µL |

Linkage specific, non-hydrolyzable tetra-ubiquitin is resistant to the activity of enzymes (DUBs) that cleave the isopeptide linkage between adjacent ubiquitin molecules. It can be used to investigate binding interactions between tetra-ubiquitin and proteins that contain elements such as ubiquitin-associated domains (UBAs), ubiquitin-interacting motifs (UIMs), and CARD domains. This product may also be useful in exploring the role of unanchored poly-ubiquitin chains in some signaling pathways.

**Di-Ub/Ub₂ (K48-linked), Agarose, human recombinant**

| UCN-315 | 25 µg |

Linkage specific, non-hydrolyzable tri-ubiquitin is resistant to the activity of enzymes (DUBs) that cleave the isopeptide linkage between adjacent ubiquitin molecules. It can be used to investigate binding interactions between tri-ubiquitin and proteins that contain elements such as ubiquitin-associated domains (UBAs) or ubiquitin-interacting motifs (UIMs). This product may also be useful in exploring the role of unanchored poly-ubiquitin chains in some signaling pathways.

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~26 kDa

**Tetra-Ub/Ub₄ (Linear), Agarose, human recombinant**

| UCN-712 | 250 µL |

This linear ubiquitin fusion protein is resistant to the activity of enzymes (DUBs) that cleave the peptide linkage between adjacent ubiquitin molecules. Ub is not expressed directly as free Ub, but rather as linear fusions either to itself or to certain ribosomal protein subunits. These Ub-fusion precursors are proteolyzed by deubiquitinating enzymes (DUBs) at the appropriate junction points to yield active Ub monomers with C-termini ending in GG. There are likely several intracellular DUBs which perform this essential processing role. This product may be useful in analyzing interactions between linear ubiquitin and proteins that contain ubiquitin-associated domains (UBAs) or ubiquitin-interacting motifs (UIMs).

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~34 kDa

**Di-Ub/Ub₂ (Linear), Agarose, human recombinant**

| UCN-710 | 100 µg |

This linear ubiquitin fusion protein is resistant to the activity of enzymes (DUBs) that cleave the peptide linkage between adjacent ubiquitin molecules. Ub is not expressed directly as free Ub, but rather as linear fusions either to itself or to certain ribosomal protein subunits. These Ub-fusion precursors are proteolyzed by deubiquitinating enzymes (DUBs) at the appropriate junction points to yield active Ub monomers with C-termini ending in GG. There are likely several intracellular DUBs which perform this essential processing role. This product may be useful in analyzing interactions between linear ubiquitin and proteins that contain ubiquitin-associated domains (UBAs) or ubiquitin-interacting motifs (UIMs).

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~34 kDa
SUMO Reagents

The small ubiquitin-related modifier (SUMO) is highly conserved and belongs to the growing family of ubiquitin-like proteins (UBLs) involved in post-translational protein modification. SUMO-1 is also known as Sentrin, GMP1, UBL1, PIC1, or SMT3. The physiological consequences of SUMOylation are distinct from that of ubiquitination. SUMO modification of proteins does not appear to target proteins for degradation but has been implicated in cell cycle progression, nuclear import, target subnuclear localization, transcriptional regulation, and the modulation of protein-protein interactions and protein stability. Although a number of SUMO substrates are cytoplasmic (RanGAP1, IκBα, GLUT1, GLUT4), it appears that SUMOylation is predominantly confined to the nucleus. These substrates include transcription factors (p53, c-Jun, c-Myc) and proteins involved in DNA regulation (topoisomerase I, PCNA).

There are three different human SUMO proteins or isoforms with distinct functions. SUMO-1 has 50% sequence identity with SUMO-2 (Sentrin-3, SMT3A) and SUMO-3 (Sentrin-2, SMT3B), while SUMO-2 and SUMO-3 genes show 95% sequence identity. SUMO-1 is usually conjugated to proteins as a monomer, while the SUMO-2 and SUMO-3 form high molecular weight polymers on proteins. SUMO proteins only have 10% primary sequence homology to ubiquitin, but they possess the characteristic ubiquitin-fold tertiary structure. They also have an unstructured N-terminal extension which most likely provides an additional interface for protein-protein interactions.

SUMOylation of target proteins is mediated by a pathway analogous to that of ubiquitination, also mediated by similar enzymes (E1, E2, E3) but with important differences. All SUMO proteins are activated by a heterodimeric E1 activating enzyme, with both subunits well conserved from yeast (Aos2/Uba2) to human (SAE1/SAE2). This ATP-dependent E1 enzyme charges the SUMO by forming a high-energy thioester intermediate which is transferred to the UbcH9 conjugating enzyme. UbcH9 is the only known E2 that is able to mediate the conjugation of SUMO (usually in the absence of an E3 ligase) to lysine residues on a variety of cellular targets. Although UbcH9 can directly recognize and modify lysines contained in a SUMOylation motif, E3-like factors most likely facilitate SUMOylation of specific substrates. SUMO precursor processing and deconjugation is catalyzed by a family of cysteine proteases known as SUMO-specific proteases (SEPs) which are distinct from the ubiquitin-specific UCHs and USPs. SEPs function in both the maturation of SUMO precursor proteins and in the removal of SUMO from modified substrates.

GST-SUMO E1 (SAE-1/UBA2), S. cerevisiae

E-310 50 µg

Conjugation of the ubiquitin-like modifier SUMO (Sentrin) requires the activities of the heterodimeric E1 (Aos1/Uba2) and the UbcH9 E2 enzyme. The dimeric activating enzyme utilizes ATP to adenylate the C-terminal glycine residue of SUMO-1 (also SUMO-2 and SUMO-3), forming a high-energy thioester bond with the cysteine residue of Uba2 and the release of AMP and PPI. The second step is the trans-esterification reaction whereby SUMO-1 is transferred to Cys of UbcH9.

Purity: ≥90% (SDS-PAGE) MW: ~114 kDa

SUMO E1 (SAE-1/UBA2), human recombinant

E-315 25 µg

Conjugation of the ubiquitin-like modifier SUMO (Sentrin) requires the activities of the heterodimeric E1 (SAE1/SAE2) and the UbcH9 E2 enzyme. The dimeric activating enzyme utilizes ATP to adenylate the C-terminal glycine residue of all SUMO proteins, forming a high-energy thioester bond with the cysteine residue of SAE2 and the concomitant release of AMP and PPI. The second step is the trans-esterification reaction whereby SUMO is transferred to Cys of UbcH9.

Purity: ≥90% (SDS-PAGE) MW: ~110 kDa

UBE2/Ubc9, human recombinant

E2-645 50 µg

UbchH is a member of the E2 family and is homologous to ubiquitin-conjugating enzymes, but is specific for the conjugation of SUMO to a variety of target proteins. This E2 is unusual in that it interacts directly with protein substrates that are modified by SUMOylation, and may play a role in substrate recognition. UbchH can mediate the conjugation of SUMO-1, SUMO-2 and SUMO-3 to a variety of proteins including RanGAP1, IκBα, and PML without the requirement of an E3 ligase.

Purity: ≥95% (SDS-PAGE) MW: ~20 kDa

SUMOylation and deSUMOylation Cycle
### SUMO REAGENTS

#### SUMO-1 Derivatives, Chains and Mutants

<table>
<thead>
<tr>
<th><strong>SUMO-1 AMC, human recombinant</strong></th>
<th>UL-651</th>
<th>50 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescent substrate for SUMO-1 hydrolyses based on the C-terminus derivatization of SUMO-1 with 7-amido-4-methylcoumarin (AMC). SUMO-1 AMC is useful for studying SUMO-1 hydrolyses when detection sensitivity or continuous monitoring of activity is essential. <strong>Purity:</strong> ≥95% (HPLC) <strong>MW:</strong> ~11.6 kDa</td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>SUMO-1 Aminoluciferin (AML), human recombinant</strong></th>
<th>UL-704</th>
<th>50 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate for SUMO deconjugating enzymes (SENPs) based on the C-terminal derivative of SUMO with aminoluciferin (AML). Rather than fluorescence as the indicator of SENP activity, SENP-labeled luciferin is processed by luciferase to give a luminescence signal. SUMO-AML not only produces a stronger signal, but also has an excellent signal-to-noise ratio over traditional fluorophores. This makes it possible to rapidly assess the activity of SENPs that poorly utilize SUMO-AMC while using much lower levels of the SENPs themselves. <strong>Purity:</strong> ≥95% (HPLC) <strong>MW:</strong> ~11.3 kDa</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>SUMO-1, human recombinant</strong></th>
<th>UL-700</th>
<th>50 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>All SUMO isoforms are translated with additional C-terminal residues that have to be removed to generate the active protein. Pro-SUMO-1 (101 amino acids) is the inactive precursor of SUMO-1 (97 amino acids) and is processed at the C-terminus by SUMO-1 specific proteases (SENPs). The resulting SUMO-1 protein has the conserved C-terminal Gly-Gly residues that function in activation and conjugation reactions. This protein can be used as a negative control in SUMOylation reactions or as a substrate for SENPs. NCBI Accession # NM_003352. <strong>Purity:</strong> ≥95% (SDS-PAGE) <strong>MW:</strong> ~11.6 kDa</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>SUMO-1 Aldehyde, human recombinant</strong></th>
<th>UL-701</th>
<th>50 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>A potent and highly specific inhibitor of SUMO-specific isopeptidases (SENPs). This protein blocks the hydrolysis of poly-SUMO chains on substrate proteins <em>in vitro</em> and thus enhances poly-SUMO chain accumulation. <strong>Purity:</strong> ≥98% (HPLC) <strong>MW:</strong> ~11.1 kDa</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>SUMO-1 Vinyl Sulfone, human recombinant</strong></th>
<th>UL-702</th>
<th>50 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>A potent, irreversible and specific inhibitor of SUMO-specific isopeptidases (SENPs). Useful for inhibiting the hydrolysis of poly-SUMO chains on substrate proteins <em>in vitro</em> and thus enhances poly-SUMO chain accumulation. <strong>Purity:</strong> ≥98% (HPLC) <strong>MW:</strong> ~11.1 kDa</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>HA-SUMO-1 Vinyl Sulfone, human recombinant</strong></th>
<th>UL-703</th>
<th>50 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>This N-terminal HA-tagged SUMO-1 is a potent, irreversible and specific inhibitor of SUMO-specific isopeptidases (SENPs). This protein inhibits the hydrolysis of poly-SUMO chains on substrate proteins <em>in vitro</em> and thus enhances poly-SUMO chain accumulation. The HA peptide sequence (YPYDVPDYA) is derived from the influenza hemagglutinin protein. This epitope allows for the sensitive identification or purification of SENP activities since it is specifically recognized by anti-HA antibodies and/or anti-HA-agarose. <strong>Purity:</strong> ≥90% (HPLC) <strong>MW:</strong> ~11.1 kDa</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>His₆-Pro-SUMO-1, human recombinant</strong></th>
<th>UL-705</th>
<th>500 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>All SUMO isoforms are translated with additional C-terminal residues that have to be removed to generate the active protein. Pro-SUMO-1 (101 amino acids) is the inactive precursor of SUMO-1 (97 amino acids) and is processed at the C-terminus by SUMO-1 specific proteases (SENPs). The resulting SUMO-1 protein has the conserved C-terminal Gly-Gly residues that function in activation and conjugation reactions. This protein can be used as a negative control in SUMOylation reactions or as a substrate for SENPs. NCBI Accession # NM_003352. <strong>Purity:</strong> ≥95% (SDS-PAGE) <strong>MW:</strong> ~12.4 kDa</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>GST-SUMO-1, human recombinant</strong></th>
<th>UL-710</th>
<th>500 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>The ubiquitin-like SUMO-1 is conjugated to a variety of proteins in the presence of UbcH9 and the SAE1/SAE2 (human) or Aos1/Uba2 (yeast) activating enzyme. SUMO-1 is derived from the precursor pro-SUMO-1 (Accession # NM_003352). Human SUMO-1 shares 46% and 47% identity with SUMO-2 and SUMO-3 respectively. SUMOylation can occur without the requirement of a specific E3 ligase activity, where SUMO is transferred directly from UbcH9 to specific substrates. SUMOylated substrates are primarily localized to the nucleus (RanGAP-1, RanBP2, PML, p53, Sp100, HIPK2) but there are also cytosolic substrates (IκBα, GLUT1, GLUT4). SUMO modification has been implicated in functions such as nuclear transport, chromosome segregation and transcriptional regulation. <strong>Purity:</strong> ≥95% (SDS-PAGE) <strong>MW:</strong> ~38.6 kDa</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>SUMO-1 K7R, human recombinant</strong></th>
<th>ULM-710</th>
<th>500 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human SUMO-1 does not contain the exact yXE consensus sequence found in SUMO-2 and SUMO-3. Within this motif y represents a large hydrophobic amino acid (I, L, or V), K is the lysine that becomes modified, X is any residue and E is glutamic acid. Many known SUMO-1 conjugation sites occur within this consensus sequence, but SUMOylation also occurs on lysine residues located within non-consensus regions. SUMO-1 has been shown to form chains <em>in vitro</em> and <em>in vivo</em> but often the linkage is uncharacterized, and the function of SUMO chains has not yet been fully elucidated. SUMO-1 multimerization <em>in vitro</em> has been shown to occur predominantly via lysines K7, K16 and K17. Mutation of lysine 7 to arginine is useful to investigate mono-SUMOylation requirements or to reduce poly-SUMO chain formation. <strong>Purity:</strong> ≥95% (SDS-PAGE) <strong>MW:</strong> ~11.1 kDa</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
SUMO-1, human recombinant

The ubiquitin-like SUMO-1 is conjugated to a variety of proteins in the presence of UbcH9 and the SAE1/SAE2 (human) or Aos1/Uba2 (yeast) activating enzyme. SUMO-1 is derived from the precursor pro-SUMO-1 (Accession # NM_003352). Human SUMO-1 shares 46% and 47% identity with SUMO-2 and SUMO-3 respectively. SUMOylation can occur without the requirement of a specific E3 ligase activity, where SUMO is transferred directly from UbcH9 to specific substrates. SUMOylated substrates are primarily localized to the nucleus (RanGAP-1, RANBP2, PML, p53, Sp100, HIPK2) but there are also cytosolic substrates (IκBα, GLUT1, GLUT4). SUMO modification has been implicated in functions such as nuclear transport, chromosome segregation and transcriptional regulation.

Purity: ≥95% (SDS-PAGE) MW: ~11.1 kDa

SUMO-1 K16R, human recombinant

Mutation of lysine 16 to arginine in SUMO-1 is useful for the analysis of poly-SUMO-1 chain formation. Human SUMO-1 does not contain the exact ψKXE motif consensus sequence found in SUMO-2 and SUMO-3 proteins, but K16 is the putative site for chain formation. SUMO-1 has been shown to form chains in vitro, but the function of SUMO chains has not yet been fully elucidated.

Purity: ≥95% (SDS-PAGE) MW: ~11.1 kDa

SUMO-1 K17R, human recombinant

Human SUMO-1 does not contain the exact ψKXE consensus sequence found in SUMO-2 and SUMO-3. Within this motif ψ represents a large hydrophobic amino acid (I, L, or V), K is the lysine that becomes modified, X is any residue and E is glutamic acid. SUMO-1 has been shown to form chains in vitro and in vivo but often the linkage is uncharacterized, and the function of SUMO chains has not yet been fully elucidated. SUMO-1 multimerization in vitro has been shown to occur predominantly via lysines K7, K16 and K17. Mutation of lysines 7 and 16 to arginine is useful to investigate mono-SUMOylation requirements or to reduce poly-SUMO chain formation.

Purity: ≥ 95% (SDS-PAGE) MW: ~11.1 kDa

His6-SUMO-1, human recombinant

The ubiquitin-like SUMO-1 is conjugated to a variety of proteins in the presence of UbcH9 and the SAE1/SAE2 (human) or Aos1/Uba2 (yeast) activating enzyme. SUMO-1 is derived from the precursor pro-SUMO-1 (Accession # NM_003352). Human SUMO-1 shares 46% and 47% identity with SUMO-2 and SUMO-3 respectively. SUMOylation can occur without the requirement of a specific E3 ligase activity, where SUMO is transferred directly from UbcH9 to specific substrates. SUMOylated substrates are primarily localized to the nucleus (RanGAP-1, RANBP2, PML, p53, Sp100, HIPK2) but there are also cytosolic substrates (IκBα, GLUT1, GLUT4). SUMO modification has been implicated in functions such as nuclear transport, chromosome segregation and transcriptional regulation.

Purity: ≥95% (SDS-PAGE) MW: ~11.9 kDa

SUMO-1 K7R, K16R, human recombinant

Human SUMO-1 does not contain the exact ψKXE consensus sequence found in SUMO-2 and SUMO-3. Within this motif ψ represents a large hydrophobic amino acid (I, L, or V), K is the lysine that becomes modified, X is any residue and E is glutamic acid. Many known SUMO-1 conjugation sites occur within this consensus sequence, but SUMOylation also occurs on lysine residues located within non-consensus regions. SUMO-1 has been shown to form chains in vitro and in vivo but often the linkage is uncharacterized, and the function of SUMO chains has not yet been fully elucidated. SUMO-1 multimerization in vitro has been shown to occur predominantly via lysines K7, K16 and K17. Mutation of lysines 7 and 17 to arginine is useful to investigate mono-SUMOylation requirements or to reduce poly-SUMO chain formation.

Purity: ≥ 95% (SDS-PAGE) MW: ~11.1 kDa

Methylated SUMO-1, human recombinant

The ubiquitin-like SUMO-1 is conjugated to a variety of proteins in the presence of UbcH9 and the SAE1/SAE2 (human) or Aos1/Uba2 (yeast) activating enzyme. SUMO-1 is derived from the precursor pro-SUMO-1 (Accession # NM_003352). Reductive methylation of lysine residues renders SUMO unable to form poly-SUMO chains via lysine linkages with other SUMO molecules. Ideal for the reduction in poly-SUMO chain length and rates of SUMO conjugation, or the demonstration of substrate mono-SUMOylation.

Purity: ≥95% (SDS-PAGE) MW: ~11.1 kDa

SUMO-1 K7R, K17R, human recombinant

Human SUMO-1 does not contain the exact ψKXE consensus sequence found in SUMO-2 and SUMO-3. Within this motif ψ represents a large hydrophobic amino acid (I, L, or V), K is the lysine that becomes modified, X is any residue and E is glutamic acid. Many known SUMO-1 conjugation sites occur within this consensus sequence, but SUMOylation also occurs on lysine residues located within non-consensus regions. SUMO-1 has been shown to form chains in vitro and in vivo but often the linkage is uncharacterized, and the function of SUMO chains has not yet been fully elucidated. SUMO-1 multimerization in vitro has been shown to occur predominantly via lysines K7, K16 and K17. Mutation of lysines 7 and 17 to arginine is useful to investigate mono-SUMOylation requirements or to reduce poly-SUMO chain formation.

Purity: ≥ 95% (SDS-PAGE) MW: ~11.1 kDa

SUMO-1 K16R, K17R, human recombinant

Human SUMO-1 does not contain the exact ψKXE consensus sequence found in SUMO-2 and SUMO-3. Within this motif ψ represents a large hydrophobic amino acid (I, L, or V), K is the lysine that becomes modified, X is any residue and E is glutamic acid. Many known SUMO-1 conjugation sites occur within this consensus sequence, but SUMOylation also occurs on lysine residues located within non-consensus regions. SUMO-1 has been shown to form chains in vitro and in vivo but often the linkage is uncharacterized, and the function of SUMO chains has not yet been fully elucidated. SUMO-1 multimerization in vitro has been shown to occur predominantly via lysines K7, K16 and K17. Mutation of lysines 7 and 17 to arginine is useful to investigate mono-SUMOylation requirements or to reduce poly-SUMO chain formation.

Purity: ≥ 95% (SDS-PAGE) MW: ~11.1 kDa
SUMO-1 K7R, K16R, K17R, human recombinant

ULM-722  250 µg

Human SUMO-1 does not contain the exact KXE consensus sequence found in SUMO-2 and SUMO-3. Within this motif w represents a large hydrophobic amino acid (I, L, or V), X is any residue and E is glutamic acid. Many known SUMO-1 conjugation sites occur within this consensus sequence, but SUMOylation also occurs on lysine residues located within non-consensus regions. SUMO-1 has been shown to form chains in vitro and in vivo but often the linkage is uncharacterized, and the function of SUMO chains has not yet been fully elucidated. SUMO-1 multimerization in vitro has been shown to occur predominantly via lysines K7, K16 and K17. Mutation of these three lysines to arginine is useful to investigate mono-SUMOylation requirements or to reduce poly-SUMO chain formation.

Purity: ≥ 95% (SDS-PAGE)  MW: ~11.1 kDa

SUMO-1 Biotin, human recombinant

UL-725  50 µg

SUMO-1 modified with biotin via primary amine coupling. This results in multiple biotinylated SUMO-1 species modified at the N-terminus, as well as at lysine residues. Although having a fully functional C-terminus, lysine modification may limit the ability of this reagent to propagate poly-SUMO chains. Biotinylated SUMO-1 can be detected using avidin-linked reagents.

Purity: ≥95% (SDS-PAGE)  MW: ~11.1 kDa

SUMO-1 Fluorescein, human recombinant

UL-735  50 µg

SUMO-1 modified with fluorescein via primary amine coupling. This results in multiple fluoresceinated SUMO-1 species modified at the N-terminus, as well as at lysine residues. Although having a fully functional C-terminus, lysine modification may limit the ability of this reagent to efficiently incorporate into poly-SUMO chains.

Purity: ≥95% (SDS-PAGE)  MW: ~11.1 kDa

SUMO-1 Rhodamine Red, human recombinant

UL-736  50 µg

SUMO-1 modified with rhodamine red via primary amine coupling resulting in modification of lysine residues as well as the N-terminus. Although having a fully functional C-terminus, lysine modification may limit the ability of this reagent to propagate poly-SUMO chains. This labeled SUMO-1 allows for direct detection spectrophotometrically with higher efficiency and sensitivity than with antibodies.

Purity: ≥95% (SDS-PAGE)  MW: ~11.1 kDa

SUMO-1 Agarose, human recombinant

UL-740  0.5 mL

SUMO-1 is covalently coupled to agarose beads via primary amines allowing for a fully functional C-terminus. Useful for affinity binding of SUMO-1 activating enzyme, SUMO conjugating E2 enzyme UbcH9, SUMO-1 ligases, SUMO C-terminal hydrolases, and other proteins/ enzymes that have an affinity for SUMO-1. The ubiquitin-like SUMO-1 is conjugated to a variety of proteins in the presence of UbcH9 and the SAE1/SAE2 activating enzyme. SUMO modification has been implicated in functions such as nuclear transport, chromosome segregation and transcriptional regulation.

SUMO-2 Derivatives, Chains and Mutants

Pro-SUMO-2, human recombinant

UL-750  500 µg

All SUMO isoforms are translated with additional C-terminal residues that have to be removed to generate the active protein. Pro-SUMO-2 (95 amino acids) is the inactive precursor of SUMO-2 (93 amino acids) and is processed at the C-terminus by SUMO-2 specific proteases (SENPs). The resulting SUMO-2 protein has the conserved C-terminal Gly-Gly residues that function in activation and conjugation reactions. This protein can be used as a negative control in SUMOylation reactions or as a substrate for SENPs. NCBI Accession # NM_006937.

Purity: ≥95% (SDS-PAGE)  MW: ~10.9 kDa

His-Pro-SUMO-2, human recombinant

UL-751  500 µg

All SUMO isoforms are translated with additional C-terminal residues that have to be removed to generate the active protein. Pro-SUMO-2 (95 amino acids) is the inactive precursor of SUMO-2 (93 amino acids) and is processed at the C-terminus by SUMO-2 specific proteases (SENPs). The resulting SUMO-2 protein has the conserved C-terminal Gly-Gly residues that function in activation and conjugation reactions. This protein can be used as a negative control in SUMOylation reactions or as a substrate for SENPs. NCBI Accession # NM_006937.

Purity: ≥95% (SDS-PAGE)  MW: ~11.7 kDa

SUMO-2, human recombinant

UL-752  500 µg

The ubiquitin-like SUMO-2 is conjugated to a variety of proteins in the presence of UbcH9 and the SAE1/SAE2 (human) or Aos1/Uba2 (yeast) activating enzyme. SUMO-2 is derived from the precursor pro-SUMO-2 (Accession # NM_006937). Human SUMO-2 shares 44% and 86% identity with SUMO-1 and SUMO-3 respectively. SUMOylation can occur without the requirement of a specific E3 ligase activity, where SUMO is transferred directly from UbcH9 to specific substrates. SUMOylated substrates are primarily localized to the nucleus (RanGAP-1, RANBP2, PML, p53, Sp100, HIPK2) but there are also cytosolic substrates (IkBα, GLUT1, GLUT4). SUMO modification has been implicated in functions such as nuclear transport, chromosome segregation, transcriptional regulation, apoptosis and protein stability.

Purity: ≥95% (SDS-PAGE)  MW: ~10.5 kDa

SUMO-2 K11R, human recombinant

ULM-752  250 µg

Mutation of lysine 11 to arginine renders SUMO-2 unable to form poly-SUMO multimers and is useful to investigate mono-SUMOylation or can be used to reduce poly-SUMO chain formation. Human SUMO-2 contains the VK11TE sequence which allows for the formation of poly-SUMO chains. K11 is the conserved lysine that becomes modified and is the point of attachment for the C-terminal glycine of the preceding SUMO-2. The function of SUMO chains has not yet been fully elucidated.

Purity: ≥95% (SDS-PAGE)  MW: ~10.6 kDa
His<sub>6</sub>-SUMO-2, human recombinant

UL-753 500 µg

The ubiquitin-like SUMO-2 is conjugated to a variety of proteins in the presence of UbcH9 and the SAE1/SAE2 (human) or Ao31/Uba2 (yeast) activating enzyme. SUMO-2 is derived from the precursor pro-SUMO-2 (Accession # NM_006937). Human SUMO-2 shares 44% and 86% identity with SUMO-1 and SUMO-3 respectively. SUMOylation can occur without the requirement of a specific E3 ligase activity, where SUMO is transferred directly from UbcH9 to specific substrates. SUMOylated substrates are primarily localized to the nucleus (RanGAP-1, RANBP2, PML, p53, Sp100, HIPK2) but there are also cytosolic substrates (IkBα, GLUT1, GLUT4). SUMO modification has been implicated in functions such as nuclear transport, chromosome segregation, transcriptional regulation, apoptosis and protein stability.

Purity: ≥95% (SDS-PAGE) MW: ~10.6 kDa

SUMO-2 Biotin, human recombinant

UL-754 50 µg

SUMO-2 modified with biotin via primary amine coupling resulting in modification of lysine residues as well as the N-terminus. Although having a fully functional C-terminus, lysine modification may limit the ability of this reagent to propagate poly-SUMO chains. Biotinylated SUMO-2 can be detected using avidin-linked reagents for higher efficiency and sensitivity than with antibodies.

Purity: ≥95% (SDS-PAGE) MW: ~10.6 kDa

SUMO-2 Agarose, human recombinant

UL-755 0.5 mL

SUMO-2 covalently coupled to agarose beads via primary amines allowing for a fully functional C-terminus. Useful for isolation and capture of SUMO-2 interacting proteins such as the SUMO activating E1 enzyme, the SUMO carrier enzyme Ubc9, SUMO E3 ligases, SENPs and other proteins/enzymes that have an affinity for SUMO proteins.

Purity: ≥95% (SDS-PAGE) MW: ~11.3 kDa

SUMO-2 Fluorescein, human recombinant

UL-756 50 µg

SUMO-2 modified with fluorescein via primary amine coupling resulting in modification of lysine residues as well as the N-terminus. Although having a fully functional C-terminus, lysine modification may limit the ability of this reagent to propagate poly-SUMO chains. This labeled SUMO-2 allows for direct detection spectrophotometrically with higher efficiency and sensitivity than with antibodies.

Purity: ≥95% (SDS-PAGE) MW: ~10.5 kDa

SUMO-2 Rhodamine Red, human recombinant

UL-757-050 50 µg

SUMO-2 modified with rhodamine red via primary amine coupling resulting in modification of lysine residues as well as the N-terminus. Although having a fully functional C-terminus, lysine modification may limit the ability of this reagent to propagate poly-SUMO chains. This labeled SUMO-2 allows for direct detection spectrophotometrically with higher efficiency and sensitivity than with antibodies.

Purity: ≥95% (SDS-PAGE) MW: ~10.6 kDa

SUMO-2 AMC, human recombinant

UL-758 50 µg

Fluorogenic substrate for SUMO-2 hydrolases based on the carboxy-terminus derivatization of SUMO-2 with 7-amido-4-methylcoumarin (AMC). SUMO-2 AMC is useful for studying SUMO-2 hydrolases (SEPNs) when detection sensitivity or continuous monitoring of activity is essential. NOTE: This protein contains an N-terminal HA-tag.

Purity: ≥95% (SDS-PAGE) MW: ~11 kDa

HA-SUMO-2 Vinyl Sulfone, human recombinant

UL-759 50 µg

This N-terminal HA-tagged SUMO is a potent, irreversible and specific inhibitor of SUMO-specific or proteases (SEPNs). This protein inhibits the hydrolysis of poly-SUMO chains on substrate proteins in vitro and thus enhances poly-SUMO chain accumulation. The HA peptide sequence (YPYDVPDYA) is derived from the influenza hemagglutinin protein. This epitope allows for the sensitive identification or purification of SENP activities since it is specifically recognized by anti-HA antibodies and/or anti-HA-agarose.

Purity: ≥90% (HPLC) MW: ~10.6 kDa

Di-SUMO-2 WT Chains, human recombinant

ULC-200 50 µg

Di-SUMO-2 is a substrate for SUMO-specific isopeptidases (SEPNs) that cleave the isopeptide linkage between two SUMO-2 molecules. It can also be used to investigate mechanism of binding and recognition by SUMO specific E1 or E2 enzymes, E3 ligases or other proteins that contain SUMO binding domains. This product is formed enzymatically with wild-type human recombinant SUMO-2 linked via lysine 11 which is the point of attachment for the C-terminal glycine of the preceding SUMO-2.

Purity: ≥95% (SDS-PAGE) MW: ~22 kDa

Poly-SUMO-2 WT Chains (SUMO-2<sub>2</sub>), human recombinant

ULC-210 25 µg

Poly-SUMO-2 chains can be used to investigate mechanisms of chain recognition, binding and hydrolysis by SUMO-specific isopeptidases (SEPNs), SUMO-specific E3 ligases or other proteins that contain SUMO-2 binding domains. This product is formed enzymatically with wild-type human recombinant SUMO-2 linked via lysine 11 which is the point of attachment for the C-terminal glycine of the preceding SUMO-2. Mono-SUMO-2 has been removed from the chain mixture.

Purity: ≥95% (SDS-PAGE)

Poly-SUMO-2 WT Chains (SUMO-2<sub>3</sub>), human recombinant

ULC-220 25 µg

Poly-SUMO-2 chains can be used to investigate mechanisms of chain recognition, binding and hydrolysis by SUMO-specific isopeptidases (SEPNs), SUMO-specific E3 ligases or other proteins that contain SUMO-2 binding domains. This product is formed enzymatically with wild-type human recombinant SUMO-2 linked via lysine 11 which is the point of attachment for the C-terminal glycine of the preceding SUMO-2. Mono- and di-SUMO-2 have been removed from the chain mixture.

Purity: ≥95% (SDS-PAGE)
SUMO REAGENTS

SUMO-3 Derivatives, Chains and Mutants

SUMO-3, human recombinant

UL-760 500 µg

All SUMO isoforms are translated with additional C-terminal residues that have to be removed to generate the active protein. Pro-SUMO-3 (103 amino acids) is the inactive precursor of SUMO-3 (92 amino acids) and is processed at the C-terminus by SUMO-3 specific proteases (SENPs). The resulting SUMO-3 protein has the conserved C-terminal Gly-Gly residues that function in activation and conjugation reactions. This protein can be used as a negative control in SUMOylation reactions or as a substrate for SENPs. NCBI Accession # NM_006936.

Purity: ≥95% (SDS-PAGE) MW: ~10.5 kDa

His<sub>6</sub>-SUMO-3, human recombinant

UL-763 500 µg

The ubiquitin-like SUMO-3 is conjugated to a variety of proteins in the presence of Ubch9 and the SAE1/SAE2 (human) or Aos1/Uba2 (yeast) activating enzyme. SUMO-3 is derived from the precursor pro-SUMO-3 (Accession # NM_006936). Human SUMO-3 shares 47% and 87% identity with SUMO-1 and SUMO-2 respectively. SUMOylation can occur without the requirement of a specific E3 ligase activity, where SUMO is transferred directly from Ubch9 to specific substrates. SUMOylated substrates are primarily localized to the nucleus (RanGAP-1, RANBP2, PML, p53, Sp100, HIPK2) but there are also cytosolic substrates (IkBa, GLUT1, GLUT4). SUMO modification has been implicated in functions such as nuclear transport, chromosome segregation, transcriptional regulation, apoptosis and protein stability.

Purity: ≥95% (SDS-PAGE) MW: ~11.4 kDa

SUMO-3 Biotin, human recombinant

UL-764 50 µg

SUMO-3 modified with biotin via primary amine coupling resulting in modification of lysine residues as well as the N-terminus. Although having a fully functional C-terminus, lysine modification may limit the ability of this reagent to propagate poly-SUMO chains. Biotinylated SUMO-3 can be detected using avidin-linked reagents for higher efficiency and sensitivity than with antibodies.

Purity: ≥95% (SDS-PAGE) MW: -10.5 kDa

SUMO-3 Agarose, human recombinant

UL-765 0.5 mL

SUMO-3 covalently coupled to agarose beads via primary amines allowing for a fully functional C-terminus. Useful for isolation and capture of SUMO-3 interacting proteins such as the SUMO activating E1 enzyme, the SUMO carrier enzyme Ubch9, SUMO E3 ligases, SENPs and other proteins/enzymes that have an affinity for SUMO proteins.

SUMO-3 Fluorescein, human recombinant

UL-766 50 µg

SUMO-3 modified with fluorescein via primary amine coupling resulting in modification of lysine residues as well as the N-terminus. Although having a fully functional C-terminus, lysine modification may limit the ability of this reagent to propagate poly-SUMO chains. This labeled SUMO-3 allows for direct detection spectrophotometrically with higher efficiency and sensitivity than with antibodies.

Purity: ≥95% (SDS-PAGE) MW: -10.5 kDa

SUMO-3 Rhodamine Red, human recombinant

UL-767 50 µg

SUMO-3 modified with rhodamine red via primary amine coupling resulting in modification of lysine residues as well as the N-terminus. Although having a fully functional C-terminus, lysine modification may limit the ability of this reagent to propagate poly-SUMO chains. This labeled SUMO-3 allows for direct detection spectrophotometrically with higher efficiency and sensitivity than with antibodies.

Purity: ≥95% (SDS-PAGE) MW: -10.5 kDa

SUMO-3 K11R, human recombinant

ULM-762 250 µg

Mutation of lysine 11 to arginine renders SUMO-3 unable to form poly-SUMO multimers and is useful to investigate mono-SUMOylation or can be used to reduce poly-SUMO chain formation. Human SUMO-3 contains the VK<sup>11TE</sup> sequence which allows for the formation of poly-SUMO chains. K<sup>11</sup> is the conserved lysine that becomes modified and is the point of attachment for the C-terminal glycine of the preceding SUMO-3. The function of SUMO chains has not yet been fully elucidated.

Purity: ≥95% (SDS-PAGE) MW: ~10.5 kDa
SUMO-3 AMC, human recombinant

UL-768 50 µg

Fluorogenic substrate for SUMO-3 hydrolases based on the carboxy-terminus derivatization of SUMO-3 with 7-amido-4-methylcoumarin (AMC). SUMO-3 AMC is useful for studying SUMO-3 hydrolases (SENP) when detection sensitivity or continuous monitoring of activity is essential. This protein contains an N-terminal HA-tag.

Purity: ≥95% (SDS-PAGE) MW: ~11 kDa

HA-SUMO-3 Vinyl Sulfone, human recombinant

UL-769 50 µg

This N-terminal HA-tagged SUMO is a potent, irreversible and specific inhibitor of SUMO-specific or proteases (SENP). This protein inhibits the hydrolysis of poly-SUMO chains on substrate proteins in vitro and thus enhances poly-SUMO chain accumulation. The HA peptide sequence (YPYDVPDYA) is derived from the influenza hemagglutinin protein. This epitope allows for the sensitive identification or purification of SENP activities since it is specifically recognized by anti-HA antibodies and/or anti-HA-agarose.

Purity: ≥90% (HPLC) MW: ~10.5 kDa

Di-SUMO-3 WT Chains, human recombinant

ULC-300 50 µg

Di-SUMO-3 is a substrate for SUMO-specific isopeptidases (SENP) that cleave the isopeptide linkage between two SUMO-3 molecules. It can also be used to investigate the mechanism of binding and recognition by SUMO specific E1 or E2 enzymes, E3 ligases or other proteins that contain SUMO binding domains. This product is formed enzymatically with wild-type human recombinant SUMO-3 linked via lysine 11 which is the point of attachment for the C-terminal glycine of the preceding SUMO-3.

Purity: ≥95% (SDS-PAGE) MW: ~22 kDa

Poly-SUMO-3 WT Chains (SUMO-3$_{2-8}$), human recombinant

ULC-310 25 µg

Poly-SUMO-3 chains can be used to investigate mechanisms of chain recognition, binding and hydrolysis by SUMO-specific isopeptidases (SENP), SUMO-specific E3 ligases or other proteins that contain SUMO-3 binding domains. This product is formed enzymatically with wild-type human recombinant SUMO-3 linked via lysine 11 which is the point of attachment for the C-terminal glycine of the preceding SUMO-3. Mono-SUMO-3 has been removed from the chain mixture.

Purity: ≥95% (SDS-PAGE)

Poly-SUMO-3 WT Chains (SUMO-3$_{3-8}$), human recombinant

ULC-320 25 µg

Poly-SUMO-3 chains can be used to investigate mechanisms of chain recognition, binding and hydrolysis by SUMO-specific isopeptidases (SENP), SUMO-specific E3 ligases or other proteins that contain SUMO-3 binding domains. This product is formed enzymatically with wild-type human recombinant SUMO-3 linked via lysine 11 which is the point of attachment for the C-terminal glycine of the preceding SUMO-3. Mono- and di-SUMO-3 have been removed from the chain mixture.

Purity: ≥95% (SDS-PAGE)

SUMO-4 Derivatives, Chains and Mutants

His$_6$-Pro-SUMO-4, human recombinant

UL-771 500 µg

All SUMO isoforms are translated with additional C-terminal residues that have to be removed to generate the active protein. His$_6$-Pro-SUMO-4 (109 amino acids) is the inactive precursor of His$_6$-SUMO-4 (107 amino acids) and is processed at the C-terminus. The resulting SUMO-4 protein has the conserved C-terminal Gly-Gly residues that function in activation and conjugation reactions. This protein can be used as a negative control in sumoylation reactions. This protein is N-terminally tagged.

Purity: >90% (SDS-PAGE) MW: ~34 kDa

SUMO Antibodies

PIAS2

AF4768 100 µg
Species: Human
Form: Antigen affinity purified sheep polyclonal
Application(s): Western blot

PIAS3

AF5120 100 µg
Species: Human
Form: Antigen affinity purified goat polyclonal
Application(s): Western blot

AF4768 100 µg
Species: Human/Mouse
Form: Antigen affinity purified goat polyclonal
Application(s): Western blot

SUMO

A-712 200 µL
Species: Human
Form: Antigen affinity purified sheep polyclonal
Application(s): Western blot

SUMO1

AF3289 100 µg
Species: Human/Mouse
Form: Antigen affinity purified goat polyclonal
Application(s): Western blot
SUMO2/3/4

AF3020 100 µg
Species: Human/Mouse
Form: Antigen affinity purified goat polyclonal
Application(s): Western blot

SUMO3

AF2959 100 µg
Species: Human/Mouse
Form: Antigen affinity purified goat polyclonal
Application(s): Western blot

MAB2959 100 µg
Species: Human
Form: Monoclonal rat IgG\textsubscript{2A}, Clone # 401513
Application(s): Immunocytochemistry

For a full listing of antibodies, please see pages 89-90.
NEDD8 is another ubiquitin-like molecule (UBL), which becomes covalently conjugated to a limited number of cellular proteins in a manner analogous to ubiquitination. Human NEDD8 shares 60% amino acid sequence identity to ubiquitin. The only known substrates of NEDD8 modification are the cullin subunits of SCF ubiquitin E3 ligases. The NEDDylation of cullins is critical for the recruitment of E2 to the ligase complex, thus facilitating ubiquitin conjugation. NEDD8 modification has therefore been implicated in cell cycle progression and cytoskeletal regulation.

As with ubiquitin and SUMO, NEDD8 is conjugated to cellular proteins after its C-terminal tail is processed. The NEDD8 activating E1 enzyme is a heterodimer composed of APPBP1 and UBA3 subunits. The APPBP1/UBA3 enzyme has homology to the N- and C-terminal halves of the ubiquitin E1 enzyme, respectively. The UBA3 subunit contains the catalytic center and activates NEDD8 in an ATP-dependent reaction by forming a high-energy thioester intermediate. The activated NEDD8 is subsequently transferred to the UbcH12 E2 enzyme, and is then conjugated to specific substrates in the presence of the appropriate E3 ligases. There are several different proteases which can remove NEDD8 from protein conjugates. UCHL1, UCHL3 and USP21 proteases have dual specificity for NEDD8 and ubiquitin. Proteases specific for NEDD8 removal are the COP9 signalosome which removes NEDD8 from the Cul1 subunit of SCF ubiquitin ligases, and NEDPI (or DEN1, SENP8).

**NEDD8 Reagents**

**GST-NEDD8 E1 (APPBP1/UBA3), human recombinant**

<table>
<thead>
<tr>
<th>E-312</th>
<th>50 µg</th>
</tr>
</thead>
</table>

The ATP-coupled activation of NEDD8 that is required for subsequent charging of the NEDD8-specific E2 UbcH12 is catalyzed by heterodimeric APPBP1-Uba3 in humans. The enzyme catalyzes the activation of the C-terminal carboxyl group of NEDD8 by forming a high-energy thioester bond in an ATP-dependent manner. Uba3 shows 43% homology to the C-terminal half of the ubiquitin activating E1 enzyme Uba1. The Uba3-catalyzed activation of NEDD8 exhibits an absolute requirement for APPBP1 which has high homology to the N-terminal half of Uba1.

**Purity:** ≥95% (SDS-PAGE) **MW:** ~135 kDa

**NEDD8 E1 (APPBP1/UBA3), human recombinant**

<table>
<thead>
<tr>
<th>E-313-025</th>
<th>25 µg</th>
</tr>
</thead>
</table>

The ATP-coupled activation of NEDD8 that is required for subsequent charging of the NEDD8-specific E2 UbcH12 is catalyzed by heterodimeric APPBP1-Uba3 in humans. The enzyme catalyzes the activation of the C-terminal carboxyl group of NEDD8 by forming a high-energy thioester bond in an ATP-dependent manner. Uba3 shows 43% homology to the C-terminal half of the ubiquitin activating E1 enzyme Uba1. The Uba3-catalyzed activation of NEDD8 exhibits an absolute requirement for APPBP1 which has high homology to the N-terminal half of Uba1.

**Purity:** ≥95% (SDS-PAGE) **MW:** ~109 kDa

**GST-UBE2M/Ubc12, human recombinant**

<table>
<thead>
<tr>
<th>E2-655</th>
<th>50 µg</th>
</tr>
</thead>
</table>

NEDDylation requires the coordinated activities of UbcH12 and the APPBP1/Uba3 activating enzyme. This E2 enzyme mediates the covalent conjugation of the ubiquitin-like protein NEDD8 to a limited number of cellular proteins such as Cdc53/Cul1 and other members of the cullin family (which are structural subunits of the Skp1-based and elongin B/C-based families of ubiquitin protein ligases). The NEDD8 pathway is essential for cell viability in fission yeast, and in mammalian cells it is essential for cell cycle progression and morphogenesis.

**Purity:** ≥95% (SDS-PAGE) **MW:** ~46 kDa

**UBE2M/Ubc12, human recombinant**

<table>
<thead>
<tr>
<th>E2-656</th>
<th>50 µg</th>
</tr>
</thead>
</table>

NEDDylation requires the coordinated activities of UbcH12 and the APPBP1/Uba3 activating enzyme. This E2 enzyme mediates the covalent conjugation of the ubiquitin-like protein NEDD8 to a limited number of cellular proteins such as Cdc53/Cul1 and other members of the cullin family (which are structural subunits of the Skp1-based and elongin B/C-based families of ubiquitin protein ligases). The NEDD8 pathway is essential for cell viability in fission yeast, and in mammalian cells it is essential for cell cycle progression and morphogenesis.

**Purity:** ≥95% (SDS-PAGE) **MW:** ~20 kDa
**His$_6$-Ube2F, human recombinant**

UL-679  50 µg  100 µg

UBE2F accepts the ubiquitin-like protein NEDD8 from the NEDD8 E1 complex and catalyzes its covalent attachment to other proteins. The specific interaction with the E3 ubiquitin ligase RBX2, but not RBX1, suggests that the RBX2-UBE2F complex modifies specific target proteins, such as CUL5. UbcH12 (UBE2M) is another NEDD8 E2 conjugating enzyme with interactions with RBX1 and targets different types of cullin substrates. The UBE2F and UBE2M enzymes thus represent a hierarchical expansion of the NEDD8 conjugation system in establishing selective culling RING ligase activation which influences substrate sub-type NEDDylation. This protein has an N-terminal His$_6$-tag.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~9 kDa

---

**NEDD8 AMC, human recombinant**

UL-652  50 µg

Fluorogenic substrate for NEDD8 hydrolases based on the carboxy-terminus derivatization of NEDD8 with 7-amido-4-methylcoumarin (AMC). NEDD8 AMC is useful for studying NEDD8 hydrolases when detection sensitivity or continuous monitoring of activity is essential.

**Purity:** ≥95% (HPLC)  **MW:** ~9 kDa

---

**NEDD8 Aminoluciferin (AML), human recombinant**

UL-804  50 µg

Substrate for deconjugating enzymes (NEDPs) based on the C-terminal derivative of NEDD8 with aminoluciferin (AML). Rather than fluorescence as the indicator of NEDP activity, NEDP liberated luciferin is processed by luciferase to give a luminescence signal. NEDD8-AML not only produces a stronger signal, but also has an excellent signal to noise ratio over traditional fluorophores. This makes it possible to rapidly assess the activity of NEDPs that poorly utilize NEDD8-AMC while using much lower levels of the NEDPs themselves.

**Purity:** ≥95% (HPLC)  **MW:** ~8.8 kDa

---

**NEDD8 Aldehyde, human recombinant**

UL-801  50 µg

A potent and highly specific inhibitor of NEDD8-specific isopeptidases (NEDP1 or SENP8). This protein blocks the hydrolysis of poly-NEDD8 chains on substrate proteins in vitro and thus enhances poly-NEDD8 chain accumulation.

**Purity:** ≥98% (HPLC)  **MW:** ~9.1 kDa

---

**NEDD8 Vinyl Sulfone, human recombinant**

UL-802  50 µg

A potent, irreversible and specific inhibitor of NEDD8-specific isopeptidases (like NEDP1 or SENP8). Useful for inhibiting the hydrolysis of poly-NEDD8 chains on substrate proteins in vitro and thus enhances poly-NEDD8 chain accumulation.

**Purity:** ≥98% (HPLC)  **MW:** ~9.1 kDa

---

**FLAG-NEDD8 Vinyl Sulfone, human recombinant**

UL-803  25 µg

This fully functional N-terminally tagged NEDD8 is a potent, irreversible inhibitor of NEDD8-specific isopeptidases (like NEDP1 or SENP8, Catalog # E-800). Useful for inhibiting the hydrolysis of poly-NEDD8 chains on substrate proteins in vitro and thus enhances poly-NEDD8 chain accumulation. The FLAG epitope, a hydrophilic octapeptide (DYKDDDDK) derived from the sequence of the bacteriophage 7 gene-10 product. This allows for the sensitive identification or purification of NEDD8 deconjugating activities since it is specifically recognized by anti-FLAG antibodies M1, M2 or M5.

**Purity:** ≥90% (HPLC)  **MW:** ~9.8 kDa

---

**Pro-NEDD8, human recombinant**

UL-810  1 mg

Protein precursor to functional NEDD8 which is ideal for the characterization of NEDD8-specific processing proteases. The ubiquitin-like protein NEDD8 is conjugated to targets by the NEDD8-specific E1 activating enzyme (APPBP1/Uba3) the UbcH12 E2 enzyme, and the ROC1/Rbx1 RING-FINGER ligase. NEDD8 plays a critical regulatory role in cell proliferation and development, and modifies nearly all members of the cullin family.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~9.1 kDa

---

**NEDD8, human recombinant**

UL-812  500 µg

The ubiquitin-like protein NEDD8 is conjugated to targets by the NEDD8-specific E1 activating enzyme (APPBP1/Uba3), the UbcH12 E2 enzyme, and the ROC1/Rbx1 RING-FINGER E3 ligase. NEDD8 plays a critical regulatory role in cell proliferation and development, and modifies nearly all members of the cullin family.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~9 kDa

---

**His$_6$-NEDD8, human recombinant**

UL-813  500 µg

The ubiquitin-like protein NEDD8 is conjugated to targets by the NEDD8-specific E1 activating enzyme (APPBP1/Uba3), the UbcH12 E2 enzyme, and the ROC1/Rbx1 RING-FINGER E3 ligase. NEDD8 plays a critical regulatory role in cell proliferation and development, and modifies nearly all members of the cullin family.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~9 kDa

---

**Methylated NEDD8, human recombinant**

UL-815  500 µg

The ubiquitin-like protein NEDD8 is conjugated to targets by the NEDD8-specific E1 activating enzyme (APPBP1/Uba3), the UbcH12 E2 enzyme, and the ROC1/Rbx1 RING-FINGER E3 ligase. NEDD8 plays a critical regulatory role in cell proliferation and development, and modifies nearly all members of the cullin family. Methylated NEDD8 is ideal for determining whether mono- or poly-NEDD8 substrate modification is required for function.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~9 kDa

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For research use only. Not for use in diagnostic procedures.
**NEDD8 Biotin, human recombinant**

UL-820 50 µg

NEDD8 modified with biotin via primary amine coupling is ideal as an alternative to radio-labeled NEDD8. This results in multiple biotinylated NEDD8 species with modified lysines as well as the N-terminus. Biotinylated NEDD8 can be detected using avidin-linked reagents. The ubiquitin-like protein NEDD8 is conjugated to targets by the NEDD8-specific E1 activating enzyme (APPBP1/Uba3), the UbcH12 E2 enzyme, and the ROC1/Rbx1 RING-FINGER E3 ligase. NEDD8 plays a critical regulatory role in cell proliferation and development, and modifies nearly all members of the cullin family.

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~8.6 kDa

**NEDD8 Fluorescein, human recombinant**

UL-830 50 µg

NEDD8 modified with fluorescein via primary amine coupling is ideal as an alternative to radiolabeled NEDD8. This results in multiple fluoresceinated NEDD8 species with modified lysines as well as the N-terminus. The ubiquitin-like protein NEDD8 is conjugated to targets by the NEDD8-specific E1 activating enzyme (APPBP1/Uba3), the UbcH12 E2 enzyme, and the ROC1/Rbx1 RING-FINGER E3 ligase. NEDD8 plays a critical regulatory role in cell proliferation and development, and modifies nearly all members of the cullin family.

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~9 kDa

**NEDD8 Rhodamine Red, human recombinant**

UL-835 50 µg

NEDD8 modified with rhodamine red via primary amine coupling resulting in modification of lysine residues as well as the N-terminus. Although having a fully functional C-terminus, lysine modification may limit the ability of this reagent to propagate poly-NEDD8 chains. This labeled NEDD8 allows for direct detection spectrophotometrically with higher efficiency and sensitivity than with antibodies.

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~12.1 kDa

**NEDD8 Agarose, human recombinant**

UL-840 0.5 mL

NEDD8 is covalently coupled to agarose beads via primary amines allowing for a fully functional C-terminus. Useful for affinity binding of NEDD8 activating E1 enzyme, the NEDD8 carrier enzyme UbcH12, NEDD8 E3 ligases, and other proteins/enzymes that have an affinity for NEDD8. The ubiquitin-like protein NEDD8 is conjugated to targets by the NEDD8-specific E1 activating enzyme (AppBp1/Uba3), the UbcH12 E2 enzyme, and the ROC1/Rbx1 RING-FINGER E3 ligase. NEDD8 plays a critical regulatory role in cell proliferation and development, and modifies nearly all members of the cullin family.

**NEDD8-13C15N, human recombinant**

UL-845 50 µg

Isotopically labeled NEDD8 is useful in determining total cellular concentrations of NEDD8 using the protein standard absolute quantification (PSAQ) or related methods. Highly purified 13C/15N labeled NEDD8 is processed for the quantitative removal of glycine and buffer salts which can interfere with chemical and in vitro reactions. The ubiquitin-like protein NEDD8 is conjugated to targets by the NEDD8-specific E1 activating enzyme (AppBp1/Uba3), the Ubc12 E2 enzyme, and the ROC1/Rbx1 RING FINGER E3 ligase. NEDD8 plays a critical regulatory role in cell proliferation and development, and modifies nearly all members of the cullin family.

**Purity:** ≥95% (SDS-PAGE)  
**MW:** ~9 kDa

**NEDD8 Antibodies**

**NEDD8**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Quantity</th>
<th>Species</th>
<th>Form</th>
<th>Application(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-Bt1</td>
<td>200 µL</td>
<td>Human</td>
<td>Antigen affinity purified sheep polyclonal</td>
<td>Western blot</td>
</tr>
<tr>
<td>AF4936</td>
<td>100 µg</td>
<td>Human/Mouse/Rat</td>
<td>Antigen affinity purified sheep polyclonal</td>
<td>Western blot, Immunohistochemistry</td>
</tr>
<tr>
<td>MAB4936</td>
<td>100 µg</td>
<td>Human/Mouse/Rat</td>
<td>Monoclonal mouse IgG&lt;sub&gt;2b&lt;/sub&gt;, Clone # 584606</td>
<td>Western blot</td>
</tr>
</tbody>
</table>

*For a full listing of antibodies, please see pages 89-90.*
ISG15 Reagents

ISG15 shares several common properties with other ubiquitin-like molecules (UBLs), but its activity is tightly regulated by specific signaling pathways that have a role in innate immunity. ISG15 was identified as an interferon stimulated gene (ISG) since its expression is induced in response to type I interferons or lipopolysaccharide treatment. Upon interferon treatment, ISG15 can be detected in both free and conjugated forms, and is secreted from monocytes and lymphocytes where it can function as a cytokine. In the cell, ISG15 co-localizes with intermediate filaments and ISGylation may modulate the JAK-STAT pathway or certain aspects of neurological disease. It is also known as UCRP (ubiquitin cross-reactive protein), since it contains 2 tandem ubiquitin homology domains and is cross-reactive with ubiquitin antibodies. In contrast to other UBLs, ISG15 has not been identified in lower eukaryotes (yeast, nematode, insects, plants) indicating its role in specialized functions.

The mechanism of ISGylation and deISGylation is similar to that of ubiquitin, although the complete system components have not yet been identified. The activating E1 enzyme (UBE1L) charges ISG15 by forming a high-energy thioester intermediate and transfers it to the UbcH8 E2 protein. UbcH8 has been identified as the major E2 for ISGylation, although it also functions in ubiquitination. The E2 protein subsequently transfers the ISG15 to specific E3 ligases and relevant intracellular substrates. Only one deconjugating protease with specificity to ISG15 has been identified to date: UBP43 (a member of the USP family) cleaves ISG15-peptide fusions and also removes ISG15 (deISGylation) from native conjugates.

**ISG15 Conjugation Cycle**

---

**GST-ISG15 E1/UBE1L, human recombinant**

**E-308**

50 µg

This E1 is responsible for the first step in ISG15-protein isopeptide bond formation. The enzyme catalyzes the activation of the C-terminal carboxyl group of ISG15 by forming a high-energy thioester bond in an ATP-dependent manner. The activated ISG15 is then transferred to UbcH8 and subsequently to lysine groups of target proteins via this thioester conjugation cascade. The E1 enzyme is a critical component for the initiation of any in vitro conjugation reactions.

**Purity:** ≥95% (SDS-PAGE) **MW:** ~138 kDa

**ISG15 E1/UBE1L, human recombinant**

**E-309**

25 µg

This E1 is responsible for the first step in ISG15-protein isopeptide bond formation. This enzyme catalyzes the activation of the C-terminal carboxyl group of ISG15 by forming a high-energy thioester bond in an ATP-dependent manner. The activated ISG15 is then transferred to UbcH8 and subsequently to lysine groups of target proteins via this thioester conjugation cascade. The E1 enzyme is a critical component for the initiation of any in vitro conjugation reactions.

**Purity:** ≥95% (SDS-PAGE) **MW:** ~112 kDa

**GST-UbcH8/UBE2L6, human recombinant**

**E2-643**

50 µg

100 µg

UbcH8 can be charged with both ubiquitin and ISG15 via the activities of the respective E1ISG and E1ub enzymes. UbcH8 is highly homologous to UbcH7, and functions in ubiquitin conjugation reactions and in HECT E3 (such as E6AP) and RING-FINGER (such as Parkin) protein mediated events. UbcH8 is the major E2 for ISG15 conjugation in reactions initiated by the ISG15-specific E1 activating enzyme. ISG15 is an ubiquitin-like protein that is conjugated to cellular proteins after IFN-α/β stimulation.

**Purity:** ≥95% (SDS-PAGE) **MW:** ~44 kDa

**UbcH8/UBE2L6, human recombinant**

**E2-644**

50 µg

100 µg

UbcH8 can be charged with both ubiquitin and ISG15 via the activities of the respective E1ISG and E1ub enzymes. UbcH8 is highly homologous to UbcH7, and functions in ubiquitin conjugation reactions and in HECT E3 (such as E6AP) and RING-FINGER (such as Parkin) protein mediated events. UbcH8 is the major E2 for ISG15 conjugation in reactions initiated by the ISG15-specific E1 activating enzyme. ISG15 is an ubiquitin-like protein that is conjugated to cellular proteins after IFN-α/β stimulation.

**Purity:** ≥95% (SDS-PAGE) **MW:** ~18 kDa
ISG15 AMC, human recombinant

UL-553 50 µg

Fluorogenic substrate for ISG15 hydrolases based on the carboxy-terminus derivatization of ISG15 with 7-amido-4-methylcoumarin (AMC). ISG15 AMC is useful for studying enzymes (such as UBP43) when detection sensitivity or continuous monitoring of activity is essential.

Purity: ≥95% (HPLC)  MW: ~17 kDa

GST-ISG15, human recombinant

UL-800 500 µg

The ubiquitin-like ISG15 is conjugated to intracellular target proteins. This pathway is distinct from that of ubiquitination with different substrate specificity and interaction with ligating enzymes. ISG15 becomes conjugated to a diverse set of proteins after IFN-α/β stimulation or microbial challenge. The functions or biochemical consequences of ISG15 conjugation to proteins are not yet known, but it appears that this modification does not target proteins for proteasomal degradation. ISG15 shows specific chemotactic activity towards neutrophils and activates them to induce release of eosinophil chemotactic factors. It may also serve as a trans-acting binding factor directing the association of ligated target proteins to intermediate filaments; and may be involved in autocrine, paracrine and endocrine mechanisms.

Purity: ≥95% (SDS-PAGE)  MW: ~42 kDa

ISG15, human recombinant

UL-601 500 µg

The ubiquitin-like ISG15 acts similarly to ubiquitin and is conjugated to intracellular target proteins. This pathway is distinct from that of ubiquitination with different substrate specificity and interaction with ligating enzymes. ISG15 becomes conjugated to a diverse set of proteins after IFN-α/β stimulation or microbial challenge. The functions or biochemical consequences of ISG15 conjugation to proteins are not yet known, but it appears that this modification does not target proteins for proteasomal degradation. ISG15 shows specific chemotactic activity towards neutrophils and activates them to induce release of eosinophil chemotactic factors. It may also serve as a trans-acting binding factor directing the association of ligated target proteins to intermediate filaments and may be involved in autocrine, paracrine and endocrine mechanisms.

Purity: ≥90% (SDS-PAGE)  MW: ~17 kDa

ISG15 Aldehyde, human recombinant

UL-602 50 µg

A potent reversible and highly specific inhibitor of ISG15-specific isopeptidases (such as UBP43). This protein blocks the hydrolysis of poly-ISG15 chains on substrate proteins in vitro and thus enhances poly-ISG15 chain accumulation.

Purity: ≥95% (HPLC)  MW: ~17 kDa

ISG15 Vinyl Sulfone, human recombinant

UL-603 50 µg

A potent irreversible and highly specific inhibitor of ISG15-specific isopeptidases (such as UBP43). This protein blocks the hydrolysis of poly-ISG15 chains on substrate proteins in vitro and thus enhances poly-ISG15 chain accumulation.

Purity: ≥95% (HPLC)  MW: ~17 kDa

ISG15 REAGENTS

www.bostonbiochem.com
**Pro-ISG15, human recombinant**

UL-615 500 µg

The ubiquitin-like ISG15 is conjugated to intracellular target proteins. This pathway is distinct from that of ubiquitination with different substrate specificities and interactions with ligating enzymes. Pro-ISG15 (165 amino acids) is the inactive precursor of ISG15 (163 amino acids). The precursor is processed at the C-terminus by an ISG15-specific protease UBP43 (also known as USP18). The mature form of ISG15 contains the conserved C-terminal di-glycine residues which are critical in activation and conjugation reactions. This protein can be used as a negative control in ISGylation reactions or as a substrate for UBP43.

**Purity:** ≥95% (SDS-PAGE) **MW:** ~17.8 kDa

**His<sub>6</sub>-Pro-ISG15, human recombinant**

UL-620 500 µg

The ubiquitin-like ISG15 is conjugated to intracellular target proteins. This pathway is distinct from that of ubiquitination with different substrate specificities and interactions with ligating enzymes. Pro-ISG15 (165 amino acids) is the inactive precursor of ISG15 (163 amino acids). The precursor is processed at the C-terminus by an ISG15-specific protease UBP43 (also known as USP18). The mature form of ISG15 contains the conserved C-terminal di-glycine residues which are critical in activation and conjugation reactions. This protein can be used as a negative control in ISGylation reactions or as a substrate for UBP43. His<sub>6</sub>-tag is on the C-terminus of the protein.

**Purity:** ≥95% (SDS-PAGE) **MW:** ~18.8 kDa

**ISG15 Agarose, human recombinant**

UL-630 0.5 mL

ISG15 covalently coupled to agarose beads via primary amines allowing for a fully functional C-terminus. Useful for isolation and capture of ISG15 interacting proteins such as the ISG15 activating enzyme, the ISG15 carrier enzyme UbcH8, ISG15 E3 ligases, UBP43 (USP18) and other proteins/ enzymes that have an affinity for ISG15 protein.

**ISG15-C78S Mutant, human recombinant**

ULM-601 250 µg

Coming soon.

**ISG15-UCRP A-600 200 µL**

**Species:** Human  
**Form:** Antigen affinity purified sheep polyclonal  
**Application(s):** Western blot

**AF4845 100 µg**

**Species:** Human  
**Form:** Antigen affinity purified goat polyclonal  
**Application(s):** Western blot

**MAB4845 100 µg**

**Species:** Human  
**Form:** Monoclonal mouse IgG<sub>2b</sub>, Clone # 539442  
**Application(s):** Western blot, Immunohistochemistry

**UBE1L/ISG15 E1 A-306 100 µL**

**Species:** Human  
**Description:** Rabbit polyclonal  
**Application(s):** Western blot, Immunohistochemistry

For a full listing of antibodies, please see pages 89-90.
UFM1 Reagents

Ubiquitin-fold Modifier 1 (UFM1) shares several common properties with ubiquitin (Ub) and other ubiquitin-like molecules (UBLs). UFM1 has a tertiary structure similar to Ub, but lacks any obvious sequence identity. It is synthesized as an inactive precursor form (pro-UFM1) which has two additional amino acids beyond the conserved glycine. The mechanism of UFM1 conjugation is similar to that of ubiquitin. Mature UFM1 has an exposed C-terminal glycine which is essential for subsequent activation by its cognate E1 protein (Uba5). This activation step results in the formation of a high-energy thioester bond in the presence of ATP. The UFM1 is subsequently transferred to its cognate E2-like enzyme (Ufc1) via a similar thioester linkage with a cysteine at the E2 active site. UFM1 is conjugated to a variety of target proteins and forms complexes with as yet unidentified proteins. Thus, presumably there exist E3 ligases (none have been identified to date) to perform the final step in UFM1 conjugation to relevant targets. The modification of proteins with UFM1 is also reversible. Two novel thiol proteases have been identified to date (UfSP1 and UfSP2) which cleave UFM1-peptide C-terminal fusions and also reverse the modification of UFM1 from native intracellular conjugates. These proteases have no obvious homology to ubiquitin deconjugating enzymes. The proteins for UFM1 conjugation (Uba5, Ufc1 and UFM1) are all conserved in metazoa and plants (but not yeast), suggesting important roles in multicellular organisms. The exact role of UFM1 modification in vivo is not yet known.

His<sub>6</sub>-UFM1 Activating Enzyme (Uba5), human recombinant

<table>
<thead>
<tr>
<th>E-319</th>
<th>25 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uba5 is the E1-like enzyme responsible for the activation of the ubiquitin-like modifier, UFM1 (Catalog # UL-500). The enzyme contains a conserved ThiF domain with a nearby cysteine catalytic residue (Cys&lt;sup&gt;250&lt;/sup&gt;) and ATP-binding motif GXGXXG. This protein is highly conserved in humans and other higher eukaryotes, but not yeast. The enzyme utilizes ATP to adenylate the C-terminal glycine residue of UFM1, forming a high-energy thioester bond with the active site cysteine residue releasing AMP. The second step is the trans-esterification reaction whereby UFM1 is transferred to the E2-like protein Ufc1 (Catalog # E2-675). This protein has an N-terminal His&lt;sub&gt;6&lt;/sub&gt;-tag.</td>
<td></td>
</tr>
</tbody>
</table>

Purity: ≥95% (SDS-PAGE)  MW: ~48 kDa

His<sub>6</sub>-Ufc1, human recombinant

<table>
<thead>
<tr>
<th>E2-675</th>
<th>50 µg</th>
<th>100 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ufc1 has been identified as the E2-like conjugating enzyme for the ubiquitin-like modifier, UFM1 (Catalog # UL-500). The protein was originally discovered in HEK293 cells by immunoprecipitation using Flag-Uba5, the E1-like activating enzyme of UFM1. Ufc1 shares no homology to other E2 enzymes except for the region around the active site cysteine (Cys&lt;sup&gt;116&lt;/sup&gt;) which is capable of forming a thioester bond with UFM1. This protein contains an N-terminal His&lt;sub&gt;6&lt;/sub&gt;-tag.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Purity: ≥95% (SDS-PAGE)  MW: ~22.6 kDa

His<sub>6</sub>-UFM1, human recombinant

<table>
<thead>
<tr>
<th>UL-500</th>
<th>250 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>UFM1 is a ubiquitin-like protein that is covalently linked to target proteins via enzymatic reactions similar to ubiquitin. UFM1 is derived from the precursor form, pro-UFM1 (Accession # NP.055701), which contains two additional amino acids (Ser&lt;sup&gt;84&lt;/sup&gt;-Cys&lt;sup&gt;85&lt;/sup&gt;) following the conserved Gly&lt;sup&gt;83&lt;/sup&gt;. While the sequence of UFM1 shares only 16% sequence identity with ubiquitin, they have similar overall tertiary structures. Mature UFM1 is activated by the E1-like enzyme Uba5 (E-319) and subsequently transferred to its cognate E2-like conjugating enzyme, Ufc1 (E2-675). It is known that UFM1 becomes conjugated to as yet unidentified proteins in HEK cells and various mouse tissues. UFM1 is conserved in metazoan and plants, but not yeast. The exact cellular function and role of UFM1 modification in vivo is not yet known. This protein contains an N-terminal His&lt;sub&gt;6&lt;/sub&gt;-tag.</td>
<td></td>
</tr>
</tbody>
</table>

Purity: ≥95% (SDS-PAGE)  MW: ~12.1 kDa
UFM1 REAGENTS

**His<sub>6</sub>-Pro UFM1, human recombinant**

UL-505 250 µg

UFM1 is a ubiquitin-like protein that is covalently linked to target proteins via enzymatic reactions similar to ubiquitin. UFM1 is derived from the precursor form pro-UFM1 (Accession # NP_057701) which contains two additional amino acids (Ser84Cys85) following the conserved Gly83. While the sequence of UFM1 shares only 16% sequence identity with ubiquitin, they have similar overall tertiary structures. Mature UFM1 is activated by the E1-like enzyme Uba5 (E-319) and subsequently transferred to its cognate E2-like conjugating enzyme, Ufc1 (E2-675). It is known that UFM1 becomes conjugated to as yet unidentified proteins in HEK cells and various mouse tissues. UFM1 is conserved in metazoas and plants, but not yeast. The exact cellular function and role of UFM1 modification in vivo is not yet known. This protein contains an N-terminal His<sub>6</sub>-tag.

**Purity:** ≥90% (SDS-PAGE)  **MW:** ~9.2 kDa

**His<sub>6</sub>-UFM1 Biotin, human recombinant**

UL-520 50 µg

UFM1 modified with biotin via primary amine coupling resulting in modification of lysine residues as well as the N-terminus. Biotinylated UFM1 can be detected using avidin-linked reagents for higher efficiency and detection sensitivity than with antibodies.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~12.1 kDa

**His<sub>6</sub>-UFM1 Fluorescein, human recombinant**

UL-521 50 µg

UFM1 modified with fluorescein via primary amine coupling, resulting in the modification of lysine residues, as well as the N-terminus. This labeled UFM1 allows for direct detection spectrophotometrically with higher efficiency and sensitivity than with antibodies. This protein contains an N-terminal His<sub>6</sub>-tag.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~12.1 kDa

**His<sub>6</sub>-UFM1 Rhodamine Red, human recombinant**

UL-522 50 µg

UFM1 modified with rhodamine red via primary amine coupling, resulting in the modification of lysine residues, as well as the N-terminus. This labeled UFM1 allows for direct detection spectrophotometrically with higher efficiency and sensitivity than with antibodies. This protein contains an N-terminal His<sub>6</sub>-tag.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~12.1 kDa

**UFM1 Agarose, human recombinant**

UL-530 0.5 mL

UFM1 covalently coupled to agarose beads via primary amines allowing for a fully functional C-terminus. Useful for isolation and capture of UFM1 interacting proteins and/or enzymes that have an affinity for this ubiquitin-like protein.

**UFM1 Antibodies**

**UFM1**

A-500 200 µL

**Species:** Human  **Form:** Protein A affinity purified rabbit polyclonal  **Application(s):** Western blot

*For a full listing of antibodies, please see pages 89-90.*
FAT10 Reagents

FAT10 is an ubiquitin-like protein (UBL) that contains two ubiquitin-like domains. It was initially identified as a gene at the major histocompatibility complex locus on human chromosome 6. FAT10 is synergistically inducible by pro-inflammatory cytokines such as TNF-α and IFN-γ. The protein contains a free C-terminal di-glycine motif that is required for the formation of FAT10 conjugates. The specific conjugating enzymes and substrates involved in this process have not yet been fully defined.

FAT10 and conjugates modified with this UBL are rapidly degraded by the 26S proteasome. The non-covalent interaction with NUB-1L enhances the binding of FAT10 conjugates to the proteasome and accelerates their degradation. FAT10 also binds non-covalently to the spindle checkpoint protein MAD2 and may play a role in spindle integrity during mitosis. Although the exact functions of FAT10 are unknown, it has been implicated in playing important roles in various cellular processes, such as cell cycle and tumorigenesis. FAT10 is up-regulated in various gastrointestinal and gynecological cancers, including hepatocellular carcinoma.

**FAT10, human recombinant**

<table>
<thead>
<tr>
<th>UL-900</th>
<th>250 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAT10 is an ubiquitin-like protein discovered by chromosomal sequencing in the human MHC class I locus. The protein consists of two ubiquitin-like domains in a head-to-tail arrangement and contains a C-terminal di-glycine which is critical for covalent conjugation to target proteins. FAT10 expression is inducible by IFN-γ and TNF-α, and it may modulate tumorigenesis through its reported interaction with the MAD2 spindle assembly checkpoint protein. Proteins conjugated to FAT10 are rapidly degraded by the 26S proteasome.</td>
<td></td>
</tr>
<tr>
<td><strong>Purity:</strong> ≥95% (SDS-PAGE) <strong>MW:</strong> ~18.5 kDa</td>
<td></td>
</tr>
</tbody>
</table>

**His₆-FAT10, human recombinant**

<table>
<thead>
<tr>
<th>UL-910</th>
<th>250 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAT10 is an ubiquitin-like protein discovered by chromosomal sequencing in the human MHC class I locus. The protein consists of two ubiquitin-like domains in a head-to-tail arrangement and contains a C-terminal di-glycine motif which is critical for covalent conjugation to target proteins. FAT10 expression is inducible by the IFN-γ and TNF-α cytokines, and it may modulate tumorigenesis through its reported interaction with the MAD2 spindle assembly checkpoint protein. Proteins conjugated to FAT10 are rapidly degraded by the proteasome.</td>
<td></td>
</tr>
<tr>
<td><strong>Purity:</strong> ≥95% (SDS-PAGE) <strong>MW:</strong> ~20.9 kDa</td>
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</table>

**FAT10 Biotin, human recombinant**

<table>
<thead>
<tr>
<th>UL-912</th>
<th>50 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAT10 modified with biotin via primary amine coupling resulting in modification of lysine residues as well as the N-terminus. Biotinylated FAT10 can be detected using avidin-linked reagents for higher efficiency and detection sensitivity than with antibodies.</td>
<td></td>
</tr>
<tr>
<td><strong>Purity:</strong> ≥95% (SDS-PAGE) <strong>MW:</strong> ~18.5 kDa</td>
<td></td>
</tr>
</tbody>
</table>

**FAT10 Fluorescein, human recombinant**

<table>
<thead>
<tr>
<th>UL-913</th>
<th>50 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAT10 modified with fluorescein via primary amine coupling, resulting in the modification of lysine residues as well as the N-terminus. This labeled FAT10 allows for direct detection spectrophotometrically with higher efficiency and sensitivity than with antibodies.</td>
<td></td>
</tr>
<tr>
<td><strong>Purity:</strong> ≥95% (SDS-PAGE) <strong>MW:</strong> ~18.5 kDa</td>
<td></td>
</tr>
</tbody>
</table>

**FAT10 Rhodamine Red, human recombinant**

<table>
<thead>
<tr>
<th>UL-914</th>
<th>50 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAT10 modified with rhodamine red via primary amine coupling resulting in modification of lysine residues as well as the N-terminus. This labeled FAT10 allows for direct detection spectrophotometrically with higher efficiency and sensitivity than with antibodies.</td>
<td></td>
</tr>
<tr>
<td><strong>Purity:</strong> ≥95% (SDS-PAGE) <strong>MW:</strong> ~18.5 kDa</td>
<td></td>
</tr>
</tbody>
</table>

**FAT10 Agarose, human recombinant**

<table>
<thead>
<tr>
<th>UL-920</th>
<th>0.5 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAT10 covalently coupled to agarose beads via primary amines allowing for a fully functional C-terminus. Useful for isolation and capture of FAT10 interacting proteins and/or enzymes that have an affinity for this ubiquitin-like protein.</td>
<td></td>
</tr>
</tbody>
</table>

**Purity:** ≥95% (SDS-PAGE) **MW:** ~18.5 kDa
Autophagy (Apg) Reagents

Bulk protein degradation and organelle clearance occurs via two major systems in eukaryotic cells. The ubiquitin proteasome pathway predominantly degrades short-lived nuclear and cytosolic proteins. The lysosomal and/or vacuolar pathway degrades larger substrates, such as protein complexes and organelles. The processing and degradation of cytoplasmic components and organelles by the lysosome and/or vacuole is known as autophagy (“self-eating”). This catabolic process is enhanced during the stress response, such as with nutrient deprivation and starvation. It thus maintains the balance between the biogenesis and production of cell organelle structures, and also destroys bacteria or aggregated proteins. Autophagy plays a role in development, aging, immunity and cell death and in humans has been associated with cancer, neurodegeneration and certain muscular diseases.

There are three primary forms of autophagy: macroautophagy, microautophagy and chaperone-mediated autophagy. During macroautophagy (the most common), the concerted actions of several different proteins lead to the formation of double-membrane sequestering vesicles (autophagosomes), followed by fusion with the lysosome or vacuole, and subsequent delivery of the inner vesicle (autophagic body) into the lumen of the degradative compartment. Hydrolytic enzymes in the lumen degrade the protein components, which are then recycled.

Autophagosome formation involves two inter-related pathways that are analogous to ubiquitin-like protein (UBL) conjugation. These pathways utilize two proteins which are the ubiquitin-like modifier proteins Apg8 and Apg12, which are conjugated to their respective substrates in conjunction with similar E1 activating and E2 conjugating enzymes. Both Apg8 and Apg12 are highly conserved in eukaryotes and in humans the Apg8 consists of a multigene family. Structurally, all Apg8 proteins have an N-terminal helical subdomain, a C-terminal domain with a conserved ubiquitin fold, and a conserved C-terminal glycine. Mammalian Apg8 consists of a multigene family. Structurally, all Apg8 proteins have an N-terminal helical subdomain, a C-terminal domain with a conserved ubiquitin fold, and a conserved C-terminal glycine. Mammalian Apg8 proteins are covalently but transiently attached to membrane lipids. Both Apg8 and Apg12 proteins are activated by Apg7, an ATP-dependent homodimeric E1 activating enzyme. This E1 enzyme charges the Apg proteins by forming a high-energy thioester intermediate which is transferred to the active site cysteine of the E2-like conjugating enzymes Apg3 (in the case of Apg8) or Apg10 (in the case of Apg12).

Apg8 proteins are covalently but transiently attached to membrane lipids. Activated Apg8 proteins eventually form conjugates with phosphatidylethanolamine (PE), which becomes a component of the complete autophagosome vesicle. Apg8 precursor processing and deconjugation is catalyzed by a novel cysteine protease, Apg4b (also known as autophagin-1). This protease removes the residue following the C-terminal glycine, which is exposed in the mature Apg8 form that is active in subsequent conjugation reactions. Apg4b also removes Apg8 proteins from PE and releases the modifier from the vesicle membrane to regenerate the soluble form.

Apg12 is synthesized in its mature form and becomes conjugated to a single substrate, Apg5 via an isopeptide linkage to a conserved lysine residue. The Apg12-Apg5 complex binds Apg16 non-covalently, and this trimer forms a large multimeric complex through self-association. The exact function of this complex is unknown, but these proteins localize at sites of autophagosome formation.

**His<sub>6</sub>-Apg8 E1 Enzyme (Apg7L/ATG7), human recombinant**

<table>
<thead>
<tr>
<th>E-317</th>
<th>25 µg</th>
</tr>
</thead>
</table>

*Autophagy is a process of bulk degradation of cytoplasmic components which are sequestered in double-membrane vesicles (autophagosomes) that deliver the contents to the lysosomal and/or vacuolar system for degradation. Apg7L is a homodimeric ubiquitin activating E1-like enzyme essential for Apg8 and Apg12 conjugation that mediates membrane fusion in autophagy. This enzyme activates the C-terminal glycine of both ubiquitin-like proteins (UBLs) Apg8 and Apg12 in an ATP-dependent manner. Its activity is essential for the conjugation of Apg8 proteins to the lipid phosphatidylethanolamine (PE), and the conjugation of Apg12L to Apg5L, and is a critical component for the initiation of any in vitro conjugation reactions.*

**Purity:** ≥95% (SDS-PAGE) **MW:** ~78.9 kDa

**His<sub>6</sub>-Apg4b/ATG4B, human recombinant**

<table>
<thead>
<tr>
<th>E-400</th>
<th>25 µg</th>
</tr>
</thead>
</table>

*Apog4b is a cysteine protease and a member of the autophagin protein family. This enzyme cleaves the C-terminal sequences of the precursor forms of Apg8 proteins (including GABARAP, GATE-16 and MAP-LC3), and regulates the lipidation and de-lipidation processes of these proteins. This specific cleavage reveals the conserved C-terminal glycine in mature and active forms of the Apg8 proteins that are conjugated to phosphatidylethanolamine (PE) during autophagy.*

**Purity:** ≥95% (SDS-PAGE) **MW:** ~46.5 kDa
Apg8p1 modified with rhodamine via primary amine coupling, resulting in the modification of lysine residues as well as the N-terminus. This labeled Apg8p1 allows for direct detection spectrophotometrically with higher efficiency and sensitivity than with antibodies. This protein contains an N-terminal His6-tag.

Purity: ≥95% (SDS-PAGE) MW: ~16 kDa

His6-GATE-16/Apg8p2 Biotin, human recombinant

UL-422 50 µg

Apg8p2 modified with biotin via primary amine coupling resulting in modification of lysine residues as well as the N-terminus. Biotinylated Apg8p2 can be detected using avidin-linked reagents for higher efficiency and detection sensitivity than with antibodies. This protein is N-terminally His6-tagged.

Purity: ≥95% (SDS-PAGE) MW: ~16.3 kDa

His6-GATE-16/Apg8p2 Fluorescein, human recombinant

UL-424 50 µg

Apg8p2 modified with fluorescein via primary amine coupling resulting in the modification of lysine residues as well as the N-terminus. This labeled Apg8p2 allows for direct detection spectrophotometrically with higher efficiency and sensitivity than with antibodies. This protein contains an N-terminal His6-tag.

Purity: ≥95% (SDS-PAGE) MW: ~15.7 kDa

GATE-16/Apg8p2 Agarose, human recombinant

UL-425 0.5 mL

GATE-16 covalently coupled to agarose beads via primary amines allowing for a fully functional C-terminus. Useful for isolation and capture of GATE-16 interacting proteins and/or enzymes that have an affinity for this ubiquitin-like protein. These include the Apg8 E1 activating enzyme Apg7, the Apg8 conjugating enzyme Apg3, Apg8 processing enzyme Apg4 and other autophagy pathway proteins and enzymes.
**His<sub>6</sub>-LC3/ MAP1LC3A/Apg8p3 Rhodamine, human recombinant**

UL-436  50 μg

Apg8p3 modified with rhodamine red via primary amine coupling, resulting in the modification of lysine residues as well as the N-terminus. This labeled Apg8p3 allows for direct detection spectrophotometrically with higher efficiency and sensitivity than with antibodies. This protein contains an N-terminal His<sub>6</sub>-tag.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~16.3 kDa

**His<sub>6</sub>-GATE-16/Apg8p2 Rhodamine, human recombinant**

UL-426  50 μg

His<sub>6</sub>-Apg8p2 modified with rhodamine red via primary amine coupling. This results in rhodamine coupled Apg8p2 species with modified lysines as well as the N-terminus. Detection of rhodamine-Apg8p2 occurs with higher efficiency and sensitivity than with antibodies. This protein contains an N-terminal His<sub>6</sub>-tag.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~15.7 kDa

**His<sub>6</sub>-LC3/ MAP1LC3A/Apg8p3 Biotin, human recombinant**

UL-430  500 μg

There are at least three groups of mammalian Apg8 proteins which are homologs of the yeast Apg8 protein, including MAP- LC3a (Microtubule-associated Protein Light Chain-3) which is part of the LC3 subfamily. The mammalian Apg8 proteins are ubiquitin-like modifiers that have divergent functions in human, and are essential in autophagic conjugation systems. This modifier protein has a conserved C-terminal glycine residue that becomes covalently attached to phosphatidylethanolamine (PE) after it is activated by the Apg7p (E1) and Apg3p (E2) enzymes. MAP-LC3 is the most abundant Apg8 homolog in autophagosomal membranes, and is thought to play a major role in mammalian autophagy.

**Purity:** ≥95% (SDS-PAGE)  **MW:** ~16.3 kDa

**HA-GABARAP/Apg8p1 AMC, human recombinant**

UL-440  25 μg

Fluorogenic substrate for Apg8-specific C-terminal hydrolases such as Apg4b (Catalog # E-400) based on the C-terminal derivatization of Apg8 with 7-amido-4-methyl-coumarin (AMC). Apg8-AMC is useful for studying such enzyme activities when detection sensitivity or continuous monitoring of activity is essential. This protein has an N-terminal HA-tag.

**Purity:** ≥90% (SDS-PAGE)  **MW:** ~14 kDa

**His<sub>6</sub>-GABARAP/Apg8p1 Vinyl Sulfone, human recombinant**

UL-441  25 μg

This N-terminal HA-tagged protein is a potent, irreversible and specific inhibitor of Apg8-specific isopeptidases (such as Apg4B, Catalog # E-400). Apg4B activities include the processing of Apg8 precursor proteins and the removal of Apg8 proteins that are conjugated to PE during autophagy. These processes can be inhibited by this VS derivative which reacts with the Apg8B active site cysteine. The HA peptide sequence (YPYDVPDYA) is derived from the influenza hemagglutinin protein. This epitope allows for the sensitive identification or purification of such deconjugating activities since it is specifically recognized by anti-HA-antibodies and/or anti-HA-agarose.

**Purity:** ≥90% (SDS-PAGE)  **MW:** ~14 kDa

**HA-GATE-16/Apg8p2 AMC, human recombinant**

UL-445  25 μg

Fluorogenic substrate for Apg8-specific C-terminal hydrolases such as Apg4b (Catalog # E-400) based on the C-terminal derivatization of Apg8 with 7-amido-4-methyl-coumarin (AMC). Apg8-AMC is useful for studying such enzyme activities when detection sensitivity or continuous monitoring of activity is essential. This protein has an N-terminal HA-tag.

**Purity:** ≥90% (SDS-PAGE)  **MW:** ~14 kDa

**His<sub>6</sub>-GATE-16/Apg8p2 AMC, human recombinant**

UL-446  25 μg

This N-terminal HA-tagged Apg8 protein is a potent, irreversible and specific inhibitor of Apg8-specific isopeptidases (such as Apg84B, Catalog # E-400). Apg84B activities include the processing of Apg8 precursor proteins and the removal of Apg8 proteins that are conjugated to phosphatidylethanolamine during autophagy. These processes can be inhibited by this vinyl sulfone derivative which reacts with the Apg84B active site cysteine. The HA peptide sequence (YPYDVPDYA) is derived from the influenza hemagglutinin protein. This epitope allows for the sensitive identification or purification of such deconjugating activities since it is specifically recognized by anti-HA-antibodies and/or anti-HA-agarose.

**Purity:** ≥90% (SDS-PAGE)  **MW:** ~13.8 kDa

**LC3/ MAP1LC3A/Apg8p3 Agarose, human recombinant**

UL-435  0.5 mL

MAP-LC3a covalently coupled to agarose beads via primary amines allowing for a fully functional C-terminus. Useful for isolation and capture of MAP-LC3a interacting proteins and/or enzymes that have an affinity for this ubiquitin-like protein. These include the Apg8 E1 activating enzyme Apg7, the Apg8 conjugating enzyme Apg3, Apg8 processing enzyme Apg4 and other autophagy pathway proteins and enzymes.

**For research use only. Not for use in diagnostic procedures.**
HA-LC3/MAP1LC3A/Agp8p3 AMC, human recombinant

UL-450 25 µg

Fluorogenic substrate for Apg8-specific C-terminal hydrolases such as Apg4b (Catalog # E-400) based on the C-terminal derivatization of Apg8 with 7-amido-4-methylcoumarin (AMC). Apg8-AMC is useful for studying such enzyme activities when detection sensitivity or continuous monitoring of activity is essential. This protein has an N-terminal HA-tag.

Purity: ≥90% (SDS-PAGE)  MW: ~14.4 kDa

HA-LC3/MAP1LC3A/Agp8p3 Vinyl Sulfone, human recombinant

UL-451 25 µg

This N-terminal HA-tagged Apg8 protein is a potent, irreversible and specific inhibitor of Apg8-specific isopeptidases (such as Apg84B, Catalog # E-400). Apg84B activities include the processing of Apg8 precursor proteins and the removal of Apg8 proteins that are conjugated to phosphatidylethanolamine during autophagy. These processes can be inhibited by this vinyl sulfone derivative which reacts with the Apg84B active site cysteine. The HA peptide sequence (YPYDVPDYA) is derived from the influenza hemagglutinin protein. This epitope allows for the sensitive identification or purification of such deconjugating activities since it is specifically recognized by anti-HA-antibodies and/or anti-HA-agarose.

Purity: ≥90% (SDS-PAGE)  MW: ~14.4 kDa

Autophagy Antibodies

ATG3

AF5450 100 µg

Species: Human/Mouse/Rat

Form: Antigen affinity purified sheep polyclonal

Application(s): Western blot

ATG4A

AF4324 100 µg

Species: Human

Form: Antigen affinity purified sheep polyclonal

Application(s): Western blot, Immunoprecipitation

ATG4B/Apg4b

MAB5279 100 µg

Species: Human

Description: Monoclonal mouse IgG1, Clone # 515803

Application(s): Western blot, Immunoprecipitation

ATG5

MAB5294 100 µg

Species: Human/Mouse/Rat

Description: Monoclonal mouse IgG1, Clone # 603813

Application(s): Western blot, Immunohistochemistry

ATG7

MAB6608 100 µg

Species: Human

Description: Monoclonal mouse IgG1, Clone # 683906

Application(s): Western blot, Immunohistochemistry

ATG10

AF5464 100 µg

Species: Human

Form: Antigen affinity purified sheep polyclonal

Application(s): Western blot

ATG12

MAB6817 100 µg

Species: Human/Mouse

Form: Monoclonal mouse IgG2b, Clone # 628902

Application(s): Western blot

For a full listing of antibodies, please see pages 89-90.
**Affinity Matrices**

**UB/UBL Interacting Affinity Matrices**

**S5a/Angiocidin Agarose, human recombinant**

AM-100 0.5 mL

S5a (Rpn10) is a component of the regulatory complex (19S) of the 26S proteasome. It binds to and recognizes poly-ubiquitinated proteins and functions as a receptor for proteins destined for proteolytic degradation. The protein recognizes Ub chains and conjugates via two Ub-interacting motif (UIM) domains. S5a has a preference for longer Ub polymers and has a low affinity for mono-, di- and tri-Ub. This affinity resin can be used for the enrichment, isolation and identification of ubiquitinated proteins, 26S substrates or proteins that contain Ub-like domains.

**S5a/Angiocidin UIM Domains Peptide Agarose, synthetic**

AM-110 0.5 mL

S5a (Rpn10) is a component of the regulatory complex (19S) of the 26S proteasome. It binds to and recognizes poly-ubiquitinated proteins and functions as a receptor for proteins destined for proteolytic degradation. The protein recognizes Ub chains and conjugates via two Ub-interacting motif (UIM) domains located at residues 211-230 (I) and 282-301 (II). Although both UIMs bind to poly-ubiquitin in vitro, UIM II has a 10-fold higher affinity for ubiquitin than UIM I. This affinity resin can be used for the enrichment, isolation and identification of ubiquitinated proteins, proteins that contain ubiquitin-like domains and/or 26S substrates.

**Ataxin UIM Domains Agarose, human recombinant**

AM-115 0.25 mL

Ataxin-3 has deubiquitinating activity in vitro. The full-length protein contains a catalytic N-terminal Josephin domain, three ubiquitin interacting motifs (UIMs), and a variable C-terminus with a polyglutamine stretch. Ataxin-3 functions as a mixed linkage, chain-editing enzyme with preferential cleavage of K63 linkages in mixed chains. Ataxin-3 also binds both K48-linked and K63-linked poly-Ub chains via its UIM domains and preferentially interacts with four or more Ub units of the K48 type. This affinity resin can be used for the enrichment, isolation and identification of K48-linked or K63-linked poly-Ub chains or ubiquitinated substrates that contain these linkages.

**RAP80 UIM Domains Agarose, human recombinant**

AM-120 0.25 mL

RAP80 (Receptor Associated Protein 80) interacts with BRCA1, a Ub E3-ligase which functions in conjugation with the BARD1 deubiquitinating enzyme. BRCA1 is recruited to DNA damage sites by poly-ubiquitin chains through RAP80, which contains 2 tandem ubiquitin-interacting motifs (UIMs). The UIM domains of RAP80 have been shown to have preferential binding to K6 and K63-linked Ub chains and binds to K48-chain with a much lower affinity. It is not known if RAP80 UIMs interact with Ub chains linked via K11, K27 or K33. RAP80 has a low affinity for mono-, di- and tri-Ub, but binds efficiently to tetra (or greater) Ub chains. This affinity resin can be used for the enrichment, isolation and identification of ubiquitinated proteins that are non-K48-linked.

**HR23A Tandem UBA (TUBE1) Agarose, human recombinant**

AM-125 0.25 mL

HR23A has two UBA domains that can each bind ubiquitin in addition to an N-terminal UBL domain that binds S5a and S2, two components of the 26S proteasome. HR23a recognizes ubiquitin through a predominantly hydrophobic surface formed by residues within α1 and α3 of each of its UBA domains. Tandem Ubiquitin Binding Entities (TUBEs) have been developed for the isolation and identification of ubiquitinated proteins. TUBEs display increased affinity for polyubiquitin moieties over the single ubiquitin binding associated domain (UBA). TUBEs also display a protective effect on polyubiquitinated proteins, allowing for detection at relatively low abundance. This affinity resin can be used for the enrichment, isolation and identification of K63-linked (preferentially) or K48-linked polyUb chains or ubiquitinated substrates that contain these linkages.

**Ubiquilin-1 Tandem UBA (TUBE2) Agarose, human recombinant**

AM-130 0.25 mL

HR23A has two UBA domains that can each bind ubiquitin in addition to an N-terminal UBL domain that binds S5a and S2, two components of the 26S proteasome. HR23a recognizes ubiquitin through a predominantly hydrophobic surface formed by residues within α1 and α3 of each of its UBA domains. Tandem Ubiquitin Binding Entities (TUBEs) have been developed for the isolation and identification of ubiquitinated proteins. TUBEs display increased affinity for polyubiquitin moieties over the single ubiquitin binding associated domain (UBA). TUBEs also display a protective effect on polyubiquitinated proteins, allowing for detection at relatively low abundance. This affinity resin can be used for the enrichment, isolation and identification of K63-linked (preferentially) or K48-linked polyUb chains or ubiquitinated substrates that contain these linkages.

**TUBEs**

Binding Entities (TUBEs) have been developed for the isolation and identification of ubiquitinated proteins. TUBEs display increased affinity for polyubiquitin moieties over the single ubiquitin binding associated domain (UBA). TUBEs also display a protective effect on polyubiquitinated proteins, allowing for detection at relatively low abundance. This affinity resin can be used for the enrichment, isolation and identification of K63-linked (preferentially) or K48-linked polyUb chains or ubiquitinated substrates that contain these linkages.

**SIM Agarose with SUMO Chains**

**SIM Agarose with Ub Chains**

A mixture of either Ub (20 µg) or SUMO (10 µg) chains was incubated with 15 µL of SIM agarose for 1.5 hours at room temperature. The resin was washed 3 times for 1 minute each with 1 mL of wash buffer. The resin was resuspended in 30 µL SDS-PAGE sample buffer and heated for 2 minutes at 37 °C. The sample was spun and 10 µL of supernatant was loaded on a 16% SDS-PAGE gel.
SUMO-interacting Motif (SIM) Peptide Agarose, synthetic

<table>
<thead>
<tr>
<th>AM-200</th>
<th>0.5 mL</th>
</tr>
</thead>
</table>

Three different amino acid consensus motifs have been identified as mediating SUMO binding and/or interaction. These SUMO-interacting motifs (SIMs) all contain a hydrophobic core sequence with a stretch of acidic amino acids either at the N- or C-terminus. Studies indicate that the SIM hydrophobic region is essential for mediating binding to the α-helix and β2-strand surfaces on SUMO proteins. The negatively charged residues surrounding the hydrophobic core can influence binding affinities and can dictate binding preferences for the various SUMO isoforms. This affinity resin is derived from a PIAS sequence, and can be used for the enrichment, isolation, and identification of SUMOylated proteins.

**His₆-Ataxin UIM Domains, human recombinant**

<table>
<thead>
<tr>
<th>UBE-100</th>
<th>250 µg</th>
</tr>
</thead>
</table>

Ataxin-3 protein belongs to a novel group of cysteine proteases similar to USP-type ubiquitin proteases and has deubiquitinating activity in vitro. The full-length protein contains an catalytic N-terminal Josephin domain, three ubiquitin interacting motifs (UIMs), and a variable C-terminus with a polyglutamine stretch. Ataxin-3 has deconjugating activity and functions as a mixed linkage, chain editing enzyme with preferential cleavage of K63 linkages in mixed chains. Ataxin-3 also binds both K48-linked and K63-linked poly-Ub chains via its UIM domains and preferentially interacts with four or more ubiquitin units. This protein can be used for the isolation and identification of K48-linked (preferentially) or K63-linked poly-Ub chains or ubiquitinated substrates that contain these linkages. This protein is His₆-tagged which allows for metal chelate affinity purification and also allows for convenient immuno-detection of conjugates using His₆-specific antibodies.

**Ataxin UIM Domains Biotin, human recombinant**

<table>
<thead>
<tr>
<th>UBE-105</th>
<th>250 µg</th>
</tr>
</thead>
</table>

Ataxin-3 protein belongs to a novel group of cysteine proteases similar to USP-type ubiquitin proteases and has deubiquitinating activity in vitro. The full-length protein contains an catalytic N-terminal Josephin domain, three ubiquitin interacting motifs (UIMs), and a variable C-terminus with a polyglutamine stretch. Ataxin-3 has deconjugating activity and functions as a mixed linkage, chain editing enzyme with preferential cleavage of K63 linkages in mixed chains. Ataxin-3 also binds both K48-linked and K63-linked poly-Ub chains via its UIM domains and preferentially interacts with four or more ubiquitin units. This protein can be used for the isolation and identification of K48-linked (preferentially) or K63-linked poly-Ub chains or ubiquitinated substrates that contain these linkages. Detection with avidin-linked reagents allows for a higher efficiency and detection sensitivity than with other antibodies.

**His₆-Ubiquilin-1 Tandem UBA (TUBE2), human recombinant**

<table>
<thead>
<tr>
<th>UBE-110</th>
<th>250 µg</th>
</tr>
</thead>
</table>

Ubiquilin-1 contains an N-terminal ubiquitin-like domain and a C-terminal ubiquitin-associated domain. It associates with proteasomes and ubiquitin ligases, and is thought to functionally link the ubiquitination machinery to the proteosome to affect in vivo protein degradation. Ubiquilin-1 has also been shown to modulate accumulation of presenilin proteins, and is found in lesions associated with Alzheimer’s and Parkinson’s disease. Tandem Ubiquitin Binding Entities (TUBEs) have been developed for the isolation and identification of ubiquitinated proteins. TUBEs display increased affinity for polyubiquitin moieties over the single ubiquitin binding associated domain (UBA). TUBEs also display a protective effect on polyubiquitinated proteins, allowing for detection at relatively low abundance. This protein can be used for the isolation and identification of K48-linked (preferentially) or K63-linked poly-Ub chains or ubiquitinated substrates that contain these linkages. This protein is His₆-tagged which allows for metal chelate affinity purification and also allows for convenient immuno-detection of conjugates using His₆-specific antibodies.

**Ubiquilin-1 Tandem UBA (TUBE2) Biotin, human recombinant**

<table>
<thead>
<tr>
<th>UBE-115</th>
<th>250 µg</th>
</tr>
</thead>
</table>

Ubiquilin-1 contains an N-terminal ubiquitin-like domain and a C-terminal ubiquitin-associated domain. It associates with proteasomes and E3 ligases, and is thought to functionally link the ubiquitination machinery to the proteosome to affect in vivo protein degradation. Ubiquilin-1 has also been shown to modulate accumulation of presenilin proteins, and is found in lesions associated with Alzheimer’s and Parkinson’s disease. Tandem Ubiquitin Binding Entities (TUBEs) have been developed for the isolation and identification of ubiquitinated proteins. TUBEs display increased affinity for polyubiquitin moieties over single ubiquitin binding associated domain (UBA). TUBEs also display a protective effect on poly ubiquitinated proteins, allowing for detection at relatively low abundance. This protein can be used for the isolation and identification of K48-linked (preferentially) or K63-linked poly-Ub chains or ubiquitinated substrates that contain these linkages. Detection with avidin-linked reagents allows for a higher efficiency and detection sensitivity than with other antibodies.

**His₆-HR23A/Rad23A Tandem UBA (TUBE1), human recombinant**

<table>
<thead>
<tr>
<th>UBE-210</th>
<th>250 µg</th>
</tr>
</thead>
</table>

HR23A has two UBA domains that can each bind ubiquitin in addition to an N-terminal UBL domain that binds S5a and S2, two components of the 26S proteosome. HR23a recognizes ubiquitin through a predominately hydrophobic surface formed by residues within α1 and α3 of each of its UBA domains. Tandem Ubiquitin Binding Entities (TUBEs) have been developed for the isolation and identification of ubiquitinated proteins. TUBEs display increased affinity for polyubiquitin moieties over the single ubiquitin binding associated domain (UBA). TUBEs also display a protective effect on poly ubiquitinated proteins, allowing for detection at relatively low abundance. This protein can be used for the enrichment, isolation and identification of K63-linked (preferentially) or K48-linked poly-Ub chains or ubiquitinated substrates that contain these linkages. This protein is His₆-tagged which allows for metal chelate affinity purification and also allows for convenient immuno-detection of conjugates using His₆-specific antibodies.
HR23A Tandem UBA (TUBE1) Biotin, human recombinant

UBE-215 250 μg

HR23A has two UBA domains that can each bind ubiquitin in addition to an N-terminal UBL domain that binds S5a and S2, two components of the 26S proteasome. HR23a recognizes ubiquitin through a predominately hydrophobic surface formed by residues within α1 and α3 of each of its UBA domains. Tandem Ubiquitin Binding Entities (TUBE1) have been developed for the isolation and identification of ubiquitinated proteins. TUBE1s display increased affinity for polyubiquitin moieties over the single ubiquitin binding associated domain (UBA). TUBE1s also display a protective effect on polyubiquitinated proteins, allowing for detection at relatively low abundance. This protein can be used for the enrichment, isolation and identification of K63-linked (preferentially) or K48-linked poly-Ub chains or ubiquitinated substrates that contain these linkages. Detection with avidin-linked reagents allows for a higher efficiency and detection sensitivity than with other antibodies.

His₅-RAP80 UIM Domains, human recombinant

UBE-230 250 μg

RAP80 (Receptor Associated Protein 80) interacts with BRCA1, a ubiquitin E3-ligase which functions in conjugation with the CARD1 deubiquitinating enzyme. BRCA1 is recruited to DNA damage sites by poly-ubiquitin chains through RAP80 which contains 2 tandem ubiquitin-interacting motifs (UIMs). RAP80 constitutes a protein complex with ABRA1 which interacts with the BRCT domain of BRCA1. Upon DNA damage the RAP80-ABRA1 complex targets the BRCA1-BARD1 complex to K6- and K63-linked poly-Ub chains at these foci. The UIM domains of RAP80 have been shown to have preferential binding to K6 and K63-linked Ub chains and binds to K48-chain with a much lower efficiency. It is not known if RAP80 UIMs interact with Ub chains linked via K11, K27 or K33. RAP80 has a low affinity for mono-, di- and tri-Ub but binds efficiently to tetra (or greater) ubiquitin chains. This protein can be used for the isolation and identification of ubiquitinated proteins that are non-K48-linked. This protein is His-tagged which allows for metal chelate affinity purification and also allows for convenient immuno-detection of conjugates using His₅-specific antibodies.

RAP80 UIM Domains Biotin, human recombinant

UBE-235 250 μg

RAP80 (Receptor Associated Protein 80) interacts with BRCA1, a ubiquitin E3-ligase which functions in conjugation with the CARD1 deubiquitinating enzyme. BRCA1 is recruited to DNA damage sites by poly-ubiquitin chains through RAP80 which contains 2 tandem ubiquitin-interacting motifs (UIMs). RAP80 constitutes a protein complex with ABRA1 which interacts with the BRCT domain of BRCA1. Upon DNA damage the RAP80-ABRA1 complex targets the BRCA1-BARD1 complex to K6- and K63-linked poly-Ub chains at these foci. The UIM domains of RAP80 have been shown to have preferential binding to K6 and K63-linked Ub chains and binds to K48-chain with a much lower efficiency. It is not known if RAP80 UIMs interact with Ub chains linked via K11, K27 or K33. RAP80 has a low affinity for mono-, di- and tri-Ub but binds efficiently to tetra (or greater) ubiquitin chains. Detection with avidin-linked reagents allows for a higher efficiency and detection sensitivity than with other antibodies.

S5a/Angiocidin Biotin, human recombinant

UBE-305 250 μg

S5a (Rpn10), a component of the 19S regulatory complex, functions as a receptor by binding to and recognizing poly-ubiquitinated proteins destined for 26S proteasome degradation. The protein recognizes ubiquitin chains and ubiquitin protein conjugates via two UIM (ubiquitin-interacting motif) domains. Detection with avidin-linked reagents allows for a higher efficiency and detection sensitivity than with other antibodies.

UB/UBL Affinity Matrices

Ubiquitin Agarose, human recombinant

U-400 1 mL
U-405 0.5 mL

Ubiquitin is covalently coupled to agarose beads via primary amines allowing for a fully functional C-terminus. Useful for affinity binding of ubiquitin activating enzyme (E1), ubiquitin carrier enzymes (E2s), ubiquitin ligases (E3s), ubiquitin C-terminal hydrolases (UCHs), and other proteins/enzymes that have an affinity for ubiquitin.

GABARAP/Apg8p1 Agarose, human recombinant

UL-415 0.5 mL

GABARAP covalently coupled to agarose beads via primary amines allowing for a fully functional C-terminus. Useful for isolation and capture of GABARAP interacting proteins and/or enzymes that have an affinity for this ubiquitin-like protein. These include the Apg8 E1 activating enzyme Apg7, the Apg8 conjugating enzyme Apg3, Apg8 processing enzyme Apg4 and other autophagy pathway proteins and enzymes.

GATE-16/Apg8p2 Agarose, human recombinant

UL-425 0.5 mL

GATE-16 covalently coupled to agarose beads via primary amines allowing for a fully functional C-terminus. Useful for isolation and capture of GATE-16 interacting proteins and/or enzymes that have an affinity for this ubiquitin-like protein. These include the Apg8 E1 activating enzyme Apg7, the Apg8 conjugating enzyme Apg3, Apg8 processing enzyme Apg4 and other autophagy pathway proteins and enzymes.

LC3/MAP1LC3A/Apg8p3 Agarose, human recombinant

UL-435 0.5 mL

MAP-LC3a covalently coupled to agarose beads via primary amines allowing for a fully functional C-terminus. Useful for isolation and capture of MAP-LC3a interacting proteins and/or enzymes that have an affinity for this ubiquitin-like protein. These include the Apg8 E1 activating enzyme Apg7, the Apg8 conjugating enzyme Apg3, Apg8 processing enzyme Apg4 and other autophagy pathway proteins and enzymes.
**SUMO-3 Agarose, human recombinant**

UL-765 0.5 mL

SUMO-3 covalently coupled to agarose beads via primary amines allowing for a fully functional C-terminus. Useful for isolation and capture of SUMO-3 interacting proteins such as the SUMO activating E1 enzyme, the SUMO carrier enzyme UbcH9, SUMO E3 ligases, SENPs and other proteins/ enzymes that have an affinity for SUMO proteins.

**NEDD8 Agarose, human recombinant**

UL-840 0.5 mL

NEDD8 is covalently coupled to agarose beads via primary amines allowing for a fully functional C-terminus. Useful for affinity binding of NEDD8 activating E1 enzyme, the NEDD8 carrier enzyme UbcH12, NEDD8 E3 ligases, and other proteins/enzymes that have an affinity for NEDD8. The ubiquitin-like protein NEDD8 is conjugated to targets by the NEDD8-specific E1 activating enzyme (AppBp1/ Uba3), the UbcH12 E2 enzyme, and the ROC1/Rbx1 RING-FINGER E3 ligase. NEDD8 plays a critical regulatory role in cell proliferation and development, and modifies nearly all members of the cullin family.

**FAT10 Agarose, human recombinant**

UL-920 0.5 mL

FAT10 covalently coupled to agarose beads via primary amines allowing for a fully functional C-terminus. Useful for isolation and capture of FAT10 interacting proteins and/or enzymes that have an affinity for this ubiquitin-like protein.
Fractions

Ubiquitin Conjugating Enzyme Fractions, *mammalian*

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-340</td>
<td>400 µg</td>
</tr>
</tbody>
</table>

The enzyme fractions supplied represent the full complement of purified conjugation enzymes (E1, E2s, and E3s) that are found in mammalian Fraction II. The enzymes have been tested and shown to work with typical [125I]-labeled substrate proteins such as lysozyme and β-lactoglobulin. These conjugation fractions contain ubiquitin C-terminal hydrolases. The addition of ubiquitin aldehyde (Catalog # U-201) is recommended for the inhibition of UCHs and to improve overall conjugate yield. The supplied fractions do not contain 20S or 26S protein degradation activity. If the substrate being conjugated requires E2 or E3 enzymes not found in Fraction II, the reaction can be supplemented with enzymes from Fraction I (Catalog # F-375). Includes complementary fractions for 2-5 conjugation reactions depending on conditions. A conjugation kit with solutions and protocol is also available (Catalog # K-960).

Protein Fraction II, *rabbit reticulocyte*

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-360</td>
<td>5 mg</td>
</tr>
</tbody>
</table>

Protein fraction from a cell extract that binds to an anion exchange resin. Essentially ubiquitin-free and ATP-free, FII contains E1, most E2s, some E3s, UCHs, and the proteasome (20S and 26S). From rabbit reticulocytes.

26S Proteasome Fraction, *mammalian*

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-365</td>
<td>200 µL</td>
</tr>
</tbody>
</table>

Mammalian 26S proteasome purified through a specific series of specialized chromatographic steps yields a partially purified fraction containing 26S proteasome and no conjugation enzymes (E1, E2s, and E3s). This stable fraction is preferred for the degradation of pre-formed ubiquitin-protein conjugates by the 26S proteasome since highly purified 26S is costly and more labile. 26S concentration is estimated based on analytical SEC at 1500 kDa and quantitation of the 20S core particle activity using a fluorogenic peptide substrate.

Protein Fraction II, *HeLa*

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-370</td>
<td>1 mg</td>
</tr>
<tr>
<td></td>
<td>5 mg</td>
</tr>
</tbody>
</table>

Protein fraction from a cell extract that binds to an anion exchange resin. Essentially ubiquitin-free and ATP-free, FII contains E1, most E2s, some E3s, UCHs, and the proteasome (20S and 26S). From *HeLa S3* cells.

S-100 Fraction, *HeLa*

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-372</td>
<td>5 mg</td>
</tr>
</tbody>
</table>

Contains the full *HeLa* cell complement of E1, E2s, E3s, 20S and 26S proteasomes, isopeptidases, and PA28. From *HeLa S3* cells.

Protein Fraction I, *HeLa*

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-375</td>
<td>1 mg</td>
</tr>
<tr>
<td></td>
<td>5 mg</td>
</tr>
</tbody>
</table>

Protein fraction of a cell extract that does not bind to anion exchange resin. Contains ubiquitin and fraction I E2 and E3 enzymes. FI is essentially free of ATP. From *HeLa S3* cells.
Protein modification by ubiquitin (Ub) is a reversible process. These deconjugation reactions are performed by specific cysteine proteases which generate monomeric Ub from a variety of C-terminal adducts. These enzymes process precursor Ub forms to their mature or active protein forms, they can process linear Ub fusion proteins, they can disassemble Ub isopeptide bonds present in poly chain species, and also remove Ub modifications from protein substrates.

Deconjugating Enzyme Set
K-E10 1 Kit
Protein modification by ubiquitin (Ub) is a reversible process. These deconjugation reactions are performed by specific cysteine proteases which generate monomeric Ub from a variety of C-terminal adducts. These enzymes process precursor Ub forms to their mature or active protein forms, they can process linear Ub fusion proteins, they can disassemble Ub isopeptide bonds present in poly chain species, and also remove Ub modifications from protein substrates.

Deconjugating Enzyme Probe Set
K­410 1 Kit
These N-terminal tagged proteins are potent, irreversible and specific inhibitors of most deconjugating enzymes including ubiquitin C-terminal hydrolases (UCHs), ubiquitin specific proteases (USPs), Apg6b, SENPs and NEDPs. They are useful for inhibiting the hydrolysis of Ub/UBL chains or Ub/UBL monomers on substrate proteins in vitro. The HA peptide sequence (YPYDVPDYA) is derived from the influenza hemagglutinin protein. This epitope allows for the sensitive identification or purification of deconjugating enzymes since it is specifically recognized by anti-HA antibodies and/or anti-HA-agarose. The FLAG epitope tag is a hydrophilic octapeptide (DYKDDDDK) derived from the sequence of the bacteriophage 7 gene-10 product. This epitope allows for the sensitive identification or purification of deconjugating activities since it is specifically recognized by anti-FLAG antibodies M1, M2 or M5.

Deconjugating Enzyme FRET Substrate Kit (K48-linked Substrates)
K-S12 1 Kit
Linkage specific diubiquitin is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. These FRET-based DUB substrates with isopeptide bonds between ubiquitin moieties are superior to gel densitometry assays. Since DUBs recognize and cleave substrates with specific steric conditions, each substrate varies in the position of the fluorophore and quencher and must be empirically tested for individual enzymes.

Deconjugating Enzyme FRET Substrate Kit (K63-linked Substrates)
K-S14 1 Kit
Linkage specific diubiquitin is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. These FRET-based DUB substrates with isopeptide bonds between ubiquitin moieties are superior to gel densitometry assays. Since DUBs recognize and cleave substrates with specific steric conditions, each substrate varies in the position of the fluorophore and quencher and must be empirically tested for individual enzymes.

Deconjugating Enzyme FRET Substrate Kit (EDANS/DABCYL Labeled Substrates)
K-S18 1 Kit
Linkage specific diubiquitin is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. These FRET-based DUB substrates with isopeptide bonds between ubiquitin moieties are superior to gel densitometry assays. Since DUBs recognize and cleave substrates with specific steric conditions, each substrate varies in the position of the fluorophore and quencher and must be empirically tested for individual enzymes.

MuRF-1 Auto-Ubiquitination Kit
K-100 1 Kit
This kit is designed to perform E3 ligase auto-ubiquitination reactions in vitro, which requires the activities of the ubiquitin E1 activating enzyme (Catalog # E-305), the E2 conjugating enzyme UbcH5c (Catalog # E2-627) and E3 ligase MuRF-1 (Catalog # E3-100). The E1 enzyme charges the ubiquitin by forming an ATP-dependent high energy thioester bond. The activated ubiquitin is subsequently transferred to UbcH5c then to the MuRF-1. The MuRF-1-S-UB complex has the ability to both auto-ubiquitinate itself and/or transfer the Ub to protein substrates (e.g. troponin). Alternatively labeled Ub proteins may be substituted for biotin-ubiquitin for detection such as fluorescein-ubiquitin (Catalog # U-590) and rhodamine-ubiquitin (Catalog # U-600). Kit contains reagents sufficient for 10 x 20 μL reactions.

Parkin Auto-Ubiquitination Kit
K-105 1 Kit
This kit is designed as a control for the conjugation of the ubiquitin to protein substrates in vitro, which requires the activities of the ubiquitin E1 activating enzyme (Catalog # E-305), the E2 conjugating enzyme UbcH7 (Catalog # E2-640) and E3 ligase Parkin (Catalog # E3-150). The E1 enzyme charges the ubiquitin by forming an ATP-dependent high energy thioester bond. The activated ubiquitin is subsequently transferred to UbcH7 then to Parkin. The Parkin-S-Ub complex has the ability to both auto-ubiquitinate and transfer the ubiquitin to various substrates. Other E2 conjugating enzymes that show activity with Parkin include UbcH8 (Catalog # E2-644) and UbcH13/Uev1a (Catalog # E2-664). Alternatively labeled ubiquitin proteins may be substituted for biotin-ubiquitin for visualization such as fluorescein-ubiquitin (Catalog # U-590) and rhodamine-ubiquitin (Catalog # U-600). Kit contains reagents sufficient for 10 x 20 μL reactions.

UFM1 Conjugation Initiation Kit
K-500 1 Kit
This kit is designed for the conjugation of the ubiquitin-like modifier UFM1 to protein substrates in vitro, which requires the activities of the human UFM1 E1 (Catalog # E-319) activating enzyme and the Ufc1 (Catalog # E2-675) E2 enzyme. The E1 enzyme charges the UFM1 by forming an ATP-dependent high energy thioester bond. The activated UFM1 is subsequently transferred to Ufc1 and this E2-S-UFM1 thioester complex can be used for the conjugation of UFM1 to protein substrates with the addition of necessary E3 enzymes (not supplied). Kit contains reagents sufficient for 10 x 20 μL reactions. All proteins are N-terminally His6-tagged.
ISG15 Conjugation Kit

K-600 1 Kit

This kit is designed for the conjugation of the ubiquitin-like modifier ISG15 to protein substrates in vitro, which requires the activities of the human ISG15 E1 activating enzyme and the UbeH9 E2 enzyme. The E1 enzyme charges the ISG15 by forming an ATP-dependent high energy thioester bond. The activated ISG15 is subsequently transferred to UbcH8 and this E2-S-Ub thioester complex can be used for the conjugation of ISG15 to protein substrates with the addition of necessary E3 enzymes (not supplied).

Kit contains reagents sufficient for 10 x 20 µL reactions.

SUMO Proteins (1, 2, 3), human recombinant

K-700 1 Kit

The ubiquitin-like SUMO-1, SUMO-2 and SUMO-3 proteins are conjugated to a variety of proteins in the presence of UbcH9 and the SAE1/SAE2 (human) or Aos1/Uba2 (yeast) activating enzyme. Human SUMO-1 shares 46% and 47% identity with SUMO-2 and SUMO-3 respectively. SUMO-1 is usually conjugated to proteins as a monomer. SUMO-2 shares 86% identity with SUMO-3 and these isoforms are known to form SUMO chains. SUMOylation can occur without the requirement of a specific E3 ligase activity, where SUMO is transferred directly from UbcH9 to specific substrates. SUMOylated substrates are primarily localized to the nucleus (RanGAP-1, RANBP2, PML, p53, Sp100, HIPK2) but there are also cytosolic substrates (IκBα, GLUT1, GLUT4, E2-25K). Unlike ubiquitination, SUMOylation does not appear to target proteins for degradation by the proteasome. SUMO modification has been implicated in diverse functions such as nuclear transport, chromosome segregation and transcriptional regulation, apoptosis and protein stability.

His₆-SUMO Proteins (1, 2, 3), human recombinant

K-705 1 Kit

The ubiquitin-like SUMO-1, SUMO-2 and SUMO-3 proteins are conjugated to a variety of proteins in the presence of UbcH9 and the SAE1/SAE2 (human) or Aos1/Uba2 (yeast) activating enzyme. Human SUMO-1 shares 46% and 47% identity with SUMO-2 and SUMO-3 respectively. SUMO-1 is usually conjugated to proteins as a monomer. SUMO-2 shares 86% identity with SUMO-3, and these isoforms are known to form SUMO chains. SUMOylation can occur without the requirement of a specific E3 ligase activity, where SUMO is transferred directly from UbcH9 to specific substrates. SUMOylated substrates are primarily localized to the nucleus (RanGAP-1, RANBP2, PML, p53, Sp100, HIPK2) but there are also cytosolic substrates (IκBα, GLUT1, GLUT4, E2-25K). Unlike ubiquitination, SUMOylation does not appear to target proteins for degradation by the proteasome. SUMO modification has been implicated in diverse functions such as nuclear transport, chromosome segregation and transcriptional regulation, apoptosis and protein stability.

Kit contains 100 µg of each protein.

SUMO-1 Mutant Protein Set, human recombinant

K-712 1 Kit

The ubiquitin-like SUMO-1, SUMO-2 and SUMO-3 proteins are conjugated to a variety of proteins in the presence of UbcH9 and the SAE1/SAE2 (human) or Aos1/Uba2 (yeast) activating enzyme. SUMO modification has been implicated in diverse functions such as nuclear transport, chromosome segregation and transcriptional regulation, apoptosis and protein stability. Human SUMO-1 shares 46% and 47% identity with SUMO-2 and SUMO-3 respectively and does not contain the exact ψKXE consensus sequence found in SUMO-2 and SUMO-3. Within this sequence ψ represents a large hydrophobic amino acid (I, L, or V), K is the lysine that becomes modified, X is any residue and E is glutamic acid. Many known SUMO-1 conjugation sites occur within this motif, but SUMOylation also occurs on lysine residues located within non-consensus regions. SUMO-1 has been shown to form chains in vitro and in vivo but often the linkage is uncharacterized, and the function of SUMO chains has not yet been fully elucidated. SUMO-1 multimerization in vitro has been shown to occur predominantly via K7, K16 and K17. The proteins in this set have these lysine residues mutated to arginine and can be used to investigate mono-SUMOylation requirements or to reduce poly-SUMO chain formation. Included is wild-type SUMO-1 to be used as a positive control in SUMO-1 conjugation assays which can be performed using the SUMO-1 Conjugation Kit (Catalog # K-710).

Kit contains 50 µg of each protein.

SUMO-2 Conjugation Kit, human recombinant

K-715 1 Kit

This kit is designed for the conjugation of the ubiquitin-like modifier SUMO-2 to protein substrates in vitro, which requires the activities of the heterodimeric human E1 activating enzyme (SAE1/SAE2) and the UbcH9 E2 enzyme.

Kit contains reagents sufficient for 10 x 20 µL reactions.

SUMO-3 Conjugation Kit, human recombinant

K-720 1 Kit

This kit is designed for the conjugation of the ubiquitin-like modifier SUMO-3 to protein substrates in vitro, which requires the activities of the heterodimeric human E1 activating enzyme (SAE1/SAE2) and the UbcH9 E2 enzyme.

Kit contains reagents sufficient for 10 x 20 µL reactions.

NEDD8 Conjugation Initiation Kit

K-800 1 Kit

This kit is designed for the conjugation of the ubiquitin-like modifier NEDD8 to protein substrates in vitro, which requires the activities of the heterodimeric human E1 activating enzyme (APPBP1/Uba3) and the UbcH12 E2 enzyme. The E1 enzyme charges the NEDD8 by forming an ATP-dependent high energy thioester bond with the active site cysteine of Uba3. The activated NEDD8 is subsequently transferred to UbcH12 and this E2-S-Ub thioester complex can be used for the conjugation of NEDD8 to protein substrates in the presence of the appropriate E3 enzymes (not supplied).

Kit contains reagents sufficient for 10 x 20 µL reactions.
**20S Proteasome Assay Kit (SDS Activation Format), rabbit**

K-900 1 Kit

This kit contains buffers and reagents for the quantitative analysis of 20S proteasome activity in cuvettes or a 96-well microtiter plate format. The 20S activity is measured by monitoring the release of free AMC from the fluorogenic peptide Suc-Leu-Leu-Val-Tyr-AMC (Catalog # S-280). The rate of AMC release may be measured either by absorbance or fluorescence over time.

Kit contains reagents sufficient for 96 x 200 µL reactions.

**S-100 Fraction Degradation Kit, HeLa**

K-910 1 Kit

This kit contains reagents to allow for the controlled degradation of exogenously added or endogenously contained substrate proteins through the ubiquitin proteasome pathway (UPP). S-100 fraction contains the full complement of all UPP cytosolic enzymes and is ideal for the demonstration of a protein being targeted by the UPP for degradation.

Kit contains reagents sufficient for 10 x 50 µL reactions.

**Fraction II Conjugation Kit, HeLa**

K-930 1 Kit

This kit contains reagents to allow for the controlled conjugation of exogenously added or endogenously contained substrate proteins through the ubiquitin proteasome pathway (UPP). Fraction II HeLa is the protein fraction of a cell extract that binds to anion exchange resin. It is essentially free of ubiquitin and ATP. FII contains E1, most E2s, some E3s, UCHs and the proteasome (20S and 26S).

Kit contains reagents sufficient for 10 x 50 µL reactions.

**S-100 Fraction Conjugation Kit, HeLa**

K-915 1 Kit

This kit contains reagents to allow for the controlled conjugation of exogenously added or endogenously contained substrate proteins through the ubiquitin proteasome pathway (UPP). S-100 fraction contains the full complement of all UPP enzymes in the cytosol and is ideal for the demonstration of a protein being targeted by the UPP for degradation.

Kit contains reagents sufficient for 10 x 50 µL reactions.

**Fraction II Degradation Kit, HeLa**

K-920 1 Kit

This kit contains reagents to allow for the controlled degradation of exogenously added or endogenously contained substrate proteins through the ubiquitin proteasome pathway (UPP). Fraction II is the protein fraction of a cell extract that binds to anion exchange resin. It contains E1, most E2s, some E3s, UCHs, and the proteasome (20S and 26S); and is essentially free of ubiquitin and ATP.

Kit contains reagents sufficient for 10 x 50 µL reactions.

**Fraction II Degradation Kit, rabbit reticulocyte**

K-925 1 Kit

This kit contains reagents to allow for the controlled degradation of exogenously added or endogenously contained substrate proteins through the ubiquitin proteasome pathway (UPP). Fraction II Reticulocyte is the protein fraction of a cell extract that binds to anion exchange resin. It is essentially free of ubiquitin and ATP and contains E1, most E2s, some E3s, UCHs and the proteasome (20S and 26S).

Kit contains reagents sufficient for 10 x 50 µL reactions.

**26S Proteasome Degradation Kit, rabbit**

K-950 1 Kit

A specific series of specialized chromatographic steps yields a partially purified fraction containing 26S proteasome and none of the conjugation enzymes (E1, E2s, or E3s). This stable fraction is preferred for the degradation of pre-formed ubiquitin-protein conjugates by the 26S proteasome since highly purified 26S is costly and more labile. The 26S concentration is estimated based on analytical SEC at 1500 kDa and quantitation of the 20S core particle activity using a fluorogenic peptide substrate.

Kit contains reagents sufficient for 10 x 50 µL reactions.

**Ubiquitin Conjugation Kit, rabbit**

K-960 1 Kit

This kit is for the formation of ubiquitinated substrate proteins. The enzymes supplied represent the full complement of purified conjugation enzymes (E1, E2s, and E3s) that are found in Fraction II. The enzymes have been tested and shown to work with typical [125I]-labeled substrate proteins such as lysozyme and β-lactoglobulin.

This conjugation fraction also contains ubiquitin C-terminal hydrolases and therefore the addition of Ubiquitin Aldehyde (Catalog # U-201) is recommended for the inhibition of UCHs to improve overall conjugate yield. The supplied fractions do not contain 20S or 26S proteasomes or any other protein degradation activity. If the substrate being conjugated requires E2 or E3 enzymes not found in Fraction II, the reaction can be supplemented with enzymes from Fraction I (Catalog # F-375).

Kit contains reagents sufficient for 10 x 50 µL reactions.

**UbcH (E2) Enzyme Set, human recombinant**

K-980 1 Kit

Set of the following E2 enzymes (10 µg of each): UbcH1, UbcH2, UbcH3, UbcH5a, UbcH5b, UbcH5c, UbcH6, UbcH7, UbcH10 and Uev1a/UbcH13 complex. Useful for selecting the appropriate enzyme in novel ubiquitination reactions.
**UbcH (E2) Enzyme Set, Dominant Negative, human recombinant**

**KITS**

**K-985**  
1 Kit

Set of the following mutant and chemically inactivated E2 enzymes: UbcH2, UbcH3, UbcH5α, UbcH6, UbcH7 and UbcH10. The mutation or chemical inactivation of the active site from cysteine to serine abolishes the ability of E2 to transfer ubiquitin to an accepting E3 protein or substrate. Useful for selecting the appropriate negative/competitive control in novel reactions.

**Ubiquitin Conjugation Initiation Kit, human recombinant**

**K-995**  
1 Kit

This kit is designed for the purpose of charging ubiquitin by E1, the first reaction in the conjugation cascade. This E1-Ubiquitin thioester can be subsequently used for the initiation of any E1 mediated reaction including E2 ubiquitin thioester formation and/or the ubiquitination conjugation reaction of target substrate proteins with the addition of E2 / E3 enzymes (not supplied). The reagents supplied allow for the thioester formation and detection of E1-S-Ub and/or E2-S-Ub with the addition of E2 enzyme (not supplied).

Kit contains reagents sufficient for 10 x 20 µL reactions.

**Solution Kits**

**Ubiquitin Conjugation Reaction Buffer Kit**

**SK-10**  
1 Kit

Ubiquitin reaction buffer kit contains the optimal buffer formulations for use in conjugation assays of ubiquitin (Catalog # U-100) to protein substrates in vitro. This requires the activities of the E1 activating enzyme (Catalog # E-305) and subsequent E2 and E3 enzymes. This buffer can be used to supplement ubiquitin thioester/conjugation initiation kit (Catalog # K-995).

**SUMO Conjugation Reaction Buffer Kit**

**SK-15**  
1 Kit

SUMO conjugation reaction buffer kit contains the optimal buffer formulations for use in assays for the conjugation of the ubiquitin-like modifiers SUMO-1 (Catalog # UL-712), SUMO-2 (Catalog # UL-752), and SUMO-3 (Catalog # UL-762), to protein substrates in vitro. These SUMO proteins are conjugated to a variety of proteins in the presence of UbcH9 (Catalog # E2-645) and SAE1/SAE2 (human) (Catalog # E-315). This kit can supplement the SUMO conjugation kits (Catalog # K-712, K-715, K-720).

**NEDD8 Conjugation Reaction Buffer Kit**

**SK-20**  
1 Kit

NEDD8 reaction buffer kit contains the optimal buffer formulations for use in assays for the conjugation of the ubiquitin-like modifier NEDD8 (Catalog # UL-812) to protein substrates in vitro. This requires the activities of the heterodimeric human E1 activating enzyme (APPBP1/Uba3) (Catalog # E-313) and the UbcH12 E2 enzyme (Catalog # E2-656). This buffer can be used to supplement NEDD8 conjugation kit (Catalog # K-800).

**ISG15 Conjugation Reaction Buffer Kit**

**SK-25**  
1 Kit

ISG15 reaction buffer kit contains the optimal buffer formulations for use in assays for the conjugation of the ubiquitin-like modifier ISG15 (Catalog # UL-601) to protein substrates in vitro, which requires the activities of the human ISG15 E1 activating enzyme (Catalog # E-308, E-309) and the UbcH8 E2 enzyme (Catalog # E2-644). This buffer can be used as a supplement to ISG15 Conjugation Kit (Catalog # K-600).

**UFM1 Conjugation Reaction Buffer Kit**

**SK-30**  
1 Kit

UFM1 reaction buffer kit contains the optimal buffer formulations for use in assays for the conjugation of the ubiquitin-like modifier UFM1 (Catalog # UL-500) to protein substrates in vitro, which requires the activities of the human UFM1 E1 activating enzyme (Catalog # E-319) and the Ufc1 E2 enzyme (Catalog # E2-675). This buffer can be used as a supplement to UFM1 Conjugation Kit (Catalog # K-500).

**Agarose Cleaning and Storage Kit**

**SK-100**  
1 Kit

Agarose cleaning and storage kit contains the optimal formulations for cleaning and storage of agarose affinity matrices. With proper cleaning and storage, these resins can be re-used for at least 5-10 applications.
## Buffers, Solutions & Standards

### Energy Regeneration Solution

<table>
<thead>
<tr>
<th>B-10</th>
<th>100 µL</th>
</tr>
</thead>
</table>

A mixture that contains MgCl₂, ATP and ATP regenerating enzymes to recycle hydrolyzed ATP (AMP, ADP) to ATP. An ideal energy source for conjugation/degradation assays that are performed with impure reagents or in crude lysates that may contain ATP-depleting activities. The amount provided is sufficient for 10 to 50 reactions depending on conditions.

### Mg-ATP Solution

<table>
<thead>
<tr>
<th>B-20</th>
<th>100 µL</th>
</tr>
</thead>
</table>

Pre-coupled Mg-ATP is an ideal energy source for semi-purified conjugation or degradation reactions. Supplied as a pH adjusted solution and will not alter reaction pH. Eliminates the reaction needed to produce usable ATP when MgCl₂ and ATP are added separately. The amount provided is sufficient for 10 to 50 reactions depending on conditions.

### 100x 20S Proteasome Activation Solution

<table>
<thead>
<tr>
<th>B-30</th>
<th>100 µL</th>
</tr>
</thead>
</table>

This buffer contains the optimal formulation for the activation of all isoforms of constitutive 20S and immuno 20S proteasome enzymes including human (Catalog # E-360), rabbit (Catalog # E-350), rat (Catalog # E-375), and mouse.

### 10X UFM1 Conjugation Reaction Buffer

<table>
<thead>
<tr>
<th>B-55</th>
<th>5 mL</th>
</tr>
</thead>
</table>

UFM1 reaction buffer contains the optimal formulation for the use in conjugation assays of the ubiquitin-like modifiers UFM1 (Catalog # UL-500), to protein substrates in vitro. The conjugation requires the activities of the human UFM1 E1 activating enzyme (Catalog # E-319) and the Ufc1 E2 enzyme (Catalog # E-675). This buffer can be used as a supplement to UFM1 Conjugation Kit (Catalog # K-500).

### 10X SUMO Conjugation Reaction Buffer

<table>
<thead>
<tr>
<th>B-60</th>
<th>5 mL</th>
</tr>
</thead>
</table>

SUMO reaction buffer contains the optimal formulation for the use in conjugation assays of the ubiquitin-like modifiers SUMO (Catalog # UL-712), SUMO-2 (Catalog # UL-752), and SUMO-3 (Catalog # UL-762), to protein substrates in vitro. These SUMO proteins are conjugated to a variety of proteins in the presence of UbcH9 (Catalog # E2-645) and SAE1/SAE2 (human) (Catalog # E-315). This buffer can be a supplement to SUMO conjugation kits (Catalog # K-712, K-715, K-720).

### 10X NEDD8 Conjugation Reaction Buffer

<table>
<thead>
<tr>
<th>B-65</th>
<th>5 mL</th>
</tr>
</thead>
</table>

NEDD8 reaction buffer contains the optimal formulation for use in conjugation assays of the ubiquitin-like modifier NEDD8 (Catalog # UL-812) to protein substrates in vitro. This requires the activities of the heterodimeric human E1 activating enzyme (APPBP1/Uba3) (Catalog # E-313) and the UbcH12 E2 enzyme (Catalog # E-656). This buffer can be used to supplement NEDD8 conjugation kit (Catalog # K-800).

### 10X Ubiquitin Conjugation Reaction Buffer

<table>
<thead>
<tr>
<th>B-70</th>
<th>5 mL</th>
</tr>
</thead>
</table>

Ubiquitin reaction buffer contains the optimal formulation for the charging of ubiquitin (Catalog # U-100) by E1 (Catalog # E-305), the first reaction in the conjugation cascade. This E1-Ubiquitin thioester can be subsequently used for the initiation of any E1 mediated reaction including E2 ubiquitin thioester formation and/or the ubiquitination conjugation reaction of target substrate proteins with the addition of E2 / E3 enzymes. This buffer can supplement the fraction II HeLa conjugation (Catalog # K-930) and fraction II reticulocyte conjugation (Catalog # K-935), ubiquitin-protein conjugation (Catalog # K-960), and ubiquitin thioester/conjugation (Catalog # K-995) kits.

### 10X ISG15 Conjugation Reaction Buffer

<table>
<thead>
<tr>
<th>B-75</th>
<th>5 mL</th>
</tr>
</thead>
</table>

ISG15 reaction buffer contains the optimal formulation for the use in assays for the conjugation of ubiquitin-like modifier ISG15 (Catalog # UL-601) to protein substrates in vitro, which requires the activities of the human ISG15 E1 activating enzyme (Catalog #s E-308, E-309) and the UbcH8 E2 enzyme (Catalog # E2-644). This buffer can be used as a supplement to the ISG15 Conjugation Kit (Catalog # K-600).

### 20X 20S Proteasome Reaction Buffer

<table>
<thead>
<tr>
<th>B-80</th>
<th>2 x 1 mL</th>
</tr>
</thead>
</table>

20S Reaction Buffer contains the optimal formulation for the quantitative analysis of constitutive 20S and immuno 20S proteasome activity. This buffer can be used as supplement to the 20S Proteasome Assay Kit (Catalog # K-900) or with any individual proteasome enzymes (Catalog #s E-350, E-360, E-370, E-375) in conjunction with the fluorogenic peptide Suc-Leu-Leu-Val-Tyr-AMC (Catalog # S-280) and 20S proteasome activation solution (Catalog # B-30) to measure activity.

### 10X Ubiquitin Reconstitution Buffer

<table>
<thead>
<tr>
<th>B-90</th>
<th>5 mL</th>
</tr>
</thead>
</table>

This buffer contains the optimal formulation for the reconstitution and subsequent dilution of lyophilized ubiquitin proteins. Refer to ubiquitin protein material data sheet for recommended concentration.

### 100X E1 Stop Buffer

<table>
<thead>
<tr>
<th>B-100</th>
<th>1 mL</th>
</tr>
</thead>
</table>

This buffer contains the optimal formulation to stop thioester/conjugation reactions dependent on E1 activating enzyme activities which are Mg-ATP dependent. Ethylenediamine tetracetic acid (EDTA) is a metal chelator that quenches activity of these Mg-ATP dependent enzymes when added to an assay in excess.
BUFFERS, SOLUTIONS AND STANDARDS

200X Conjugation Stop Buffer

B-105 0.5 mL

This buffer contains the optimal formulation for the general inhibition of enzyme activities that conjugate ubiquitin and ubiquitin-like (UBL) protein (SUMO, NEDD8, ISG15) to protein substrates. Useful during the preparation of crude lysate samples to enrich for protein conjugates or to quench in vitro assays. This agent will also quench the activities of cysteine proteases.

200X Deconjugation Stop Buffer

B-110 0.5 mL

This buffer contains the optimal formulation for the general inhibition of enzyme activities that deconjugate ubiquitin and ubiquitin-like (UBL) protein (SUMO, NEDD8, ISG15) from protein substrates. Useful during the preparation of crude lysate samples to enrich for protein conjugates or to quench in vitro assays. This agent will also quench the activities of other non-related cysteine proteases. More specific deconjugating enzyme inhibitors include aldehyde and vinyl sulfone derivatives such as ubiquitin (Catalog #s U-201, U-202), SUMO (Catalog #s UL-701, UL-702), NEDD8 (Catalog #s UL-801, UL-802) and ISG15 (Catalog #s UL-602, UL-603).

5X Loading Buffer

B-125 1 mL

Loading buffer for separation and visualization proteins with SDS-PAGE and Western blot analysis. Sample can be prepared under reducing conditions with the addition of a reducing agent such as β-mercaptoethanol or DTT.

5X Agarose Storage Buffer

B-130 1 mL

Agarose storage buffer contains the optimal conditions for storage of agarose affinity matrices. It is recommended that this product is used in conjunction with agarose cleaning buffer (Catalog # B-135) and agarose wash buffer (Catalog # B-140). This product can also supplement the agarose cleaning and storage kit (Catalog # SK-10).

5X Agarose Cleaning Buffer

B-135 1 mL

Agarose cleaning buffer contains the optimal conditions for cleaning agarose affinity matrices. It is recommended that this product is used in conjunction with agarose wash buffer (Catalog # B-140) and agarose storage buffer (Catalog # B-130). This product can also supplement the agarose cleaning and storage kit (Catalog # SK-10).

5X Agarose Wash Buffer

B-140 1 mL

Agarose wash buffer contains the optimal conditions for the washing of agarose affinity matrices. It is recommended that this product is used in conjunction with agarose cleaning buffer (Catalog # B-135) and agarose storage buffer (Catalog # B-130). This product can also supplement the agarose cleaning and storage kit (Catalog # SK-10).

AMC Standard

B-200 100 µL

A fluorogenic standard useful for quantitating assays monitoring amino-4-methylcoumarin (AMC) release. These include ubiquitin-AMC (Catalog # U-550), SUMO-1-AMC (Catalog # UL-551), NEDD8-AMC (Catalog # UL-552), ISG15-AMC (Catalog # UL-555), and proteasome substrates (Catalog #s. S-220, S-230, S-260, S-280). The concentration of the AMC released can be calculated from a standard curve prepared with AMC standards.

AFC Standard

B-210 100 µL

A fluorogenic standard useful for quantitating assays monitoring 7-amino-4-trifluoromethylcoumarin (AFC) release such as those utilizing ubiquitin-AFC (Catalog # U-551). The concentration of the AFC released can be calculated from a standard curve prepared with AFC standards.
### Antibodies

<table>
<thead>
<tr>
<th>E1 Activating Enzymes:</th>
<th>Catalog #</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATG7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UBE1L/ISG15 E1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>E2 Conjugating Enzymes:</th>
<th>Catalog #</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATG3</td>
<td>AF5450</td>
<td>100 µg</td>
</tr>
<tr>
<td>ATG10</td>
<td>AF5464</td>
<td>100 µg</td>
</tr>
<tr>
<td>UbeH1/UBE2K/E2‑25K</td>
<td>A-603</td>
<td>200 µL</td>
</tr>
<tr>
<td>UbeH2/UBE2H</td>
<td>A-605</td>
<td>200 µL</td>
</tr>
<tr>
<td>UbeH3/Cdc34</td>
<td>A-610</td>
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</tr>
<tr>
<td>UbeH5/UBE2D</td>
<td>A-615</td>
<td>200 µL</td>
</tr>
<tr>
<td>UbeH6/UBE2E1</td>
<td>A-630</td>
<td>200 µL</td>
</tr>
<tr>
<td>UbeH7/UBE2L3</td>
<td>A-640</td>
<td>200 µL</td>
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<tr>
<td>UbeH9/UBE2E3</td>
<td>A-645</td>
<td>200 µL</td>
</tr>
<tr>
<td>UbeH10/UBE2C</td>
<td>A-650</td>
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</tr>
<tr>
<td>UbeH12/UBE2M</td>
<td>A-655</td>
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</tr>
<tr>
<td>UBE2K/E2‑25K rabbit polyclonal</td>
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<td>100 µg</td>
</tr>
<tr>
<td>UBE2K/E2‑25K monoclonal mouse IgG&lt;sub&gt;2a&lt;/sub&gt;</td>
<td>MAB6609</td>
<td>100 µg</td>
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</table>

<table>
<thead>
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<th>E3 Ligase Enzymes:</th>
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<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1 C-Terminus sheep polyclonal</td>
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<tr>
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<td>MAB2210</td>
<td>100 µg</td>
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<tr>
<td>BRCA1 N-Terminus goat polyclonal</td>
<td>AF2210</td>
<td>100 µg</td>
</tr>
<tr>
<td>Phospho-BRCA1 (S1423) rabbit polyclonal</td>
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<td>100 µg</td>
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<td>100 µg</td>
</tr>
<tr>
<td>CBL monoclonal mouse IgG&lt;sub&gt;2a&lt;/sub&gt;</td>
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<tr>
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<tr>
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<td>AF7217</td>
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<table>
<thead>
<tr>
<th>Substrate Proteins:</th>
<th>Catalog #</th>
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</tr>
</thead>
<tbody>
<tr>
<td>p53 goat polyclonal</td>
<td>AF1355</td>
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<tr>
<td>p53 biotinylated goat polyclonal</td>
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<td>p53 agarose-conjugated goat polyclonal</td>
<td>GAF1355</td>
<td>250 µL</td>
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<td>p53 HRP-conjugated goat polyclonal</td>
<td>HAF1355</td>
<td>100 µL</td>
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<td>p53 monoclonal mouse IgG&lt;sub&gt;2a&lt;/sub&gt;</td>
<td>MAB1355</td>
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<td>p53 monoclonal mouse IgG&lt;sub&gt;2a&lt;/sub&gt;</td>
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<td>Catalog #</td>
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<td>-----------</td>
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</tr>
<tr>
<td><strong>Ubiquitin:</strong></td>
<td></td>
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</tr>
<tr>
<td>Ubiquitin rabbit polyclonal</td>
<td>A-100</td>
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<td>Ubiquitin&lt;sup&gt;+&lt;/sup&gt;1 rabbit polyclonal</td>
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<td>BAM7032</td>
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<td><strong>SUMO:</strong></td>
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<td>PIAS2 sheep polyclonal</td>
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<td>100 µg</td>
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<td>SUMO3 monoclonal rat IgG&lt;sub&gt;1a&lt;/sub&gt;</td>
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<td>NEDD8 sheep polyclonal</td>
<td>A-812</td>
<td>200 µL</td>
</tr>
<tr>
<td>NEDD8 sheep polyclonal</td>
<td>AF4936</td>
<td>100 µg</td>
</tr>
<tr>
<td>NEDD8 monoclonal mouse IgG&lt;sub&gt;m&lt;/sub&gt;</td>
<td>MAB4936</td>
<td>100 µg</td>
</tr>
<tr>
<td><strong>ISG15:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISG15/UCRP sheep polyclonal</td>
<td>A-600</td>
<td>200 µL</td>
</tr>
<tr>
<td>ISG15/UCRP goat polyclonal</td>
<td>AF4845</td>
<td>100 µg</td>
</tr>
<tr>
<td>ISG15/UCRP monoclonal mouse IgG&lt;sub&gt;m&lt;/sub&gt;</td>
<td>MAB4845</td>
<td>100 µg</td>
</tr>
<tr>
<td>UBEI/ISG15 E1 rabbit polyclonal</td>
<td>A-306</td>
<td>100 µL</td>
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<tr>
<td><strong>UFM1:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UFM1 rabbit polyclonal</td>
<td>A-500</td>
<td>200 µL</td>
</tr>
<tr>
<td><strong>Autophagy:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATG3 sheep polyclonal</td>
<td>AF5450</td>
<td>100 µg</td>
</tr>
<tr>
<td>ATG4A sheep polyclonal</td>
<td>AF4324</td>
<td>100 µg</td>
</tr>
<tr>
<td>ATG4A monoclonal mouse IgG&lt;sub&gt;m&lt;/sub&gt;</td>
<td>MAB4324</td>
<td>100 µg</td>
</tr>
<tr>
<td>ATG4B/Apg4b monoclonal mouse IgG&lt;sub&gt;1&lt;/sub&gt;</td>
<td>MAB5279</td>
<td>100 µg</td>
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<tr>
<td>ATG5 monoclonal mouse IgG&lt;sub&gt;m&lt;/sub&gt;</td>
<td>MAB5294</td>
<td>100 µg</td>
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<td>ATG7 monoclonal mouse IgG&lt;sub&gt;1&lt;/sub&gt;</td>
<td>MAB6608</td>
<td>100 µg</td>
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<td>ATG10 sheep polyclonal</td>
<td>AF5464</td>
<td>100 µg</td>
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<td>ATG12 monoclonal mouse IgG&lt;sub&gt;m&lt;/sub&gt;</td>
<td>MAB6807</td>
<td>100 µg</td>
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<td><strong>Fusion Tag:</strong></td>
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<tr>
<td>Avidin-HRP conjugated hen egg white</td>
<td>A-115</td>
<td>200 µL</td>
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<tr>
<td>GST/S. japonicum Glutathione-S-Transferase goat polyclonal</td>
<td>A-30</td>
<td>200 µL</td>
</tr>
</tbody>
</table>
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