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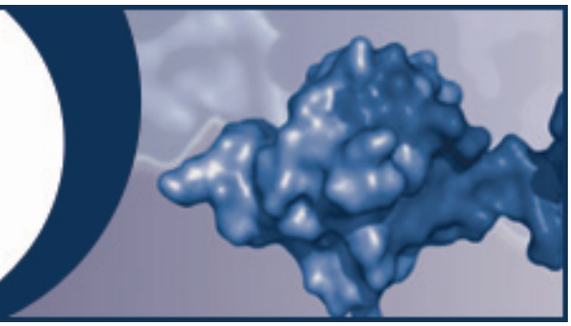

NEW PRODUCTS
May 2013

E3 Ligase Kits

MuRF1/S5a Ubiquitination Kit

K-102 1 Kit

MuRF1 (Muscle-specific RING-finger protein 1) is a RING-finger E3 ligase found in striated muscle (heart and skeletal) and iris tissues. The protein contains both RING and B-box zinc fingers, and a coiled-coil tripartite fold known as TRIM. MuRF1 ligase activity regulates the proteasomal degradation of cardiac troponin 1 and other sarcomeric-associated proteins. This kit is designed for in vitro MuRF1-mediated ubiquitination of user-supplied substrates such as cardiac troponin 1, MYH7, or muscle creatine kinase. Ubiquitinated proteins can be used in downstream applications, or analyzed by Western blot using antibodies specific for the target protein. A control substrate, S5a (SP-400) and detection antibody (AF5540) are included in the kit.

MDM2/p53 Ubiquitination Kit, version B

K-200B 1 Kit

The RING-finger ubiquitin E3 ligase MDM2 (Murine Double Minute 2) is an important regulator of the tumor suppressor protein p53. MDM2 ligase activity is at least partially responsible for the ubiquitination and subsequent proteasomal degradation of p53. This kit is designed for in vitro ubiquitination of p53 (SP-450) by MDM2. The ubiquitinated protein can be detected by Western blot using the supplied α-p53 monoclonal antibody (MAB1355). This kit can be used as a positive control in studies that monitor the activities of other known or putative E3 ligase enzymes that ubiquitinate p53. Ubiquitinated p53 substrate can also be used to study E4 ligase activities of enzymes such as UBE4B, or the activity of deubiquitinating enzymes such as USP7 (E-518)

MDM2/S5a Ubiquitination Kit

K-210 1 Kit

The RING-finger ubiquitin E3 ligase MDM2 (Murine Double Minute 2) is an important regulator of the tumor suppressor protein p53. MDM2 ligase activity is at least partially responsible for the ubiquitination and subsequent proteasomal degradation of p53 and other proteins. This kit is designed for in vitro MDM2-mediated ubiquitination of user-supplied substrates such as p53 (SP-450), β-Arrestin, and DYRK2. Ubiquitinated proteins can be used in downstream applications, or analyzed by Western blot using antibodies specific for the target protein. A control substrate, S5a (SP-400) and detection antibody (AF5540) are included in the kit.

RNF4/di-SUMO3 Ubiquitination Kit

K-220 1 Kit

"RNF4 (SNURF) is a RING-finger ubiquitin E3 ligase that ubiquitinates and mediates the proteasomal destruction of PML/RARα, a fusion protein that is a hallmark of acute promyelocytic leukemia (APL). APL can sometimes be treated effectively with arsenic trioxide, which induces PML/RARα modification by small ubiquitin-like modifiers (SUMO). RNF4 subsequently binds to and ubiquitinates the poly-SUMOylated PML/RARα, thereby targeting it to the proteasome.
RNF4 contains four SUMO-interacting motifs (SIMs) that function to recruit this ligase to a variety of poly-sumoylated substrates. RNF4 will autoubiquitinate in vitro, and will also ubiquitinate poly-SUMO chains. This kit is designed for in vitro RNF4-mediated ubiquitination of user-supplied, poly-SUMOylated substrates such as PML, PEA3, CENP1, and PARP1. The resulting proteins can be used in downstream applications, or analyzed by Western blot using antibodies specific for the target protein. A control substrate, di-SUMO3 (ULC-300) and detection antibody (MAB2959) are included in the kit."

E6AP/S5a Ubiquitination Kit

K-230 1 Kit

E6AP (E6-Associated Protein) is a HECT domain ubiquitin E3 ligase that ubiquitinates and mediates the proteasomal destruction of substrate proteins. HECT domain ligases use an active site cysteine to accept charged ubiquitin from ubiquitin-E2 thioester complexes for subsequent transfer to substrate proteins; in this way HECT class ligases are distinct from most RING class ligases in that the latter facilitate transfer of ubiquitin from charged E2's directly to substrate proteins without an E3-ubiquitin thioester intermediate. This kit is designed for in vitro E6AP-mediated ubiquitination of user-supplied, reported substrates such as β-Arrestin, and DYRK2. Ubiquitinated proteins can be used in downstream applications, or analyzed by Western blot using antibodies specific for the target protein. A control substrate, S5a (SP-400) and detection antibody (AF5540) are included in the kit.

E6AP/E6/p53 Ubiquitination Kit

K-240 1 Kit

E6AP (E6-Associated Protein) (E3-230) is a HECT domain ubiquitin E3 ligase that ubiquitinates and mediates the proteasomal destruction of substrate proteins. HECT domain ligases use an active site cysteine to accept charged ubiquitin from ubiquitin-E2 thioester complexes for subsequent transfer to substrate proteins; in this way HECT class ligases are distinct from most RING class ligases in that the latter facilitate transfer of ubiquitin from charged E2's directly to substrate proteins without an E3-ubiquitin thioester intermediate. E6 (Early protein 6) (AP-120) is a viral protein produced in cells infected with the Human Papillomavirus. E6 forms a complex with the host cell E6AP generating a ligase activity that polyubiquitinates tumor suppressors p53 and p73 and targets them to the 26S proteasome for degradation. As a result DNA damage and chromosomal instabilities increase, often leading to cell proliferation and cancer. The E6/E6AP complex also targets other substrates for ubiquitination, such as TERT, BAK1, FADD, and pro-CASP8—none of which appear to be substrates for E6AP in the absence of E6. This kit is designed for in vitro E6AP/E6-mediated ubiquitination of user-supplied substrates. Ubiquitinated proteins can be used in downstream applications, or analyzed by Western blot using antibodies specific for the target protein. A control substrate, His6-FLAG-p53 (SP-452) and detection antibody (MAB1355) are included in the kit.

CHIP/Luciferase Ubiquitination Kit

K-280 1 Kit

"CHIP (Carboxy terminus of HSP70-Interacting Protein) is a U-Box ubiquitin E3 ligase that ubiquitinates and mediates the proteasomal destruction of misfolded chaperone substrates. CHIP functions in coordination with several chaperone complexes, including HSP40, HSP70, and HSP90. CHIP activity may be modulated by the deubuiquitinase Ataxin-3, which restricts the length of ubiquitin chains attached to CHIP substrates and prevents further chain extension. This kit is designed for in vitro ubiquitination of user-supplied substrates by CHIP or the CHIP/HSP70/HSP40 ternary complex. Ubiquitinated proteins can be used in downstream applications, or analyzed by Western blot using antibodies specific for the target protein. A luciferase control substrate and detection antibody are included in the kit.
NOTE: Kit contains reagents sufficient for 10 x 30 µl reactions and 5 Western Blots (mini-gel format)."

E3 Ligases

Ubiquitin E3 Ligase Ring Finger 4 (RNF4), *human recombinant*

E3-210 50 µg

RNF4 (small nuclear ring finger protein, SNURF) is a RING-finger ubiquitin E3 ligase that ubiquitinates and mediates the proteasomal destruction of targets such as PML, PEA3, CENP1, and PARP1. In addition to the RING domain, RNF4 contains four SUMO-interacting motifs (SIMs) that function to recruit this ligase to poly-sumoylated substrates. RNF4 will autoubiquitinate in vitro, and will also ubiquitinate poly-SUMO chains. This recombinant protein is untagged.

Purity: > 95% by SDS-PAGE **MW:** 21 kDa

Ubiquitin E3 Ligase CHIP (Stub1), *human recombinant*

E3-220 50 µg

CHIP (Carboxy terminus of Hsp70-Interacting Protein) is a U-Box ubiquitin E3 ligase that ubiquitinates and mediates the proteasomal destruction of misfolded chaperone substrates. CHIP functions in coordination with several chaperone complexes, including Hsp40, Hsp70, and Hsp90. CHIP activity may be modulated by the deubuiquitinase Ataxin-3, which restricts the length of ubiquitin chains attached to CHIP substrates and prevents further chain extension. This protein contains a C-terminal 6-His tag.

Purity: > 90% by SDS-PAGE **MW:** 36 kDa

Ubiquitin E3 Ligase E6AP (UBE3A), *human recombinant*

E3-230 50 µg

E6AP (E6-Associated Protein) is a HECT domain ubiquitin E3 ligase that ubiquitinates and mediates the proteasomal destruction of substrate proteins. HECT domain ligases use an active site cysteine to accept charged ubiquitin from ubiquitin-E2 thioester complexes for subsequent transfer to substrate proteins; in this way HECT class ligases are distinct from most RING class ligases in that the latter facilitate transfer of ubiquitin from charged E2's directly to substrate proteins without an E3-ubiquitin thioester intermediate. The viral HPV-E6 protein (present in cells infected with human papillomavirus) forms a complex with E6AP to generate a ligase activity that polyubiquitinates the tumor suppressors p53 and p73 and targets them to the 26S proteasome for degradation. This protein contains a C-terminal 6-His tag.

Purity: > 95% by SDS-PAGE **MW:** 102 kDa

Non-hydrolyzable Chains

Non-hydrolyzable di-Ub (K11-linked)

UCN-40 25 µg

Linkage specific, non-hydrolyzable di-ubiquitin is resistant to the activity of enzymes (DUB's) 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). Biological roles of K11-linked polyubiquitin are less understood than K48- or K63-linked Ub chains, though recent evidence points to a role for K11 linkages in the degradation of the anaphase-promoting complex (APC/C), and in TNF -stimulated NF- B activation.

Purity: > 95 % by SDS-PAGE **MW:** 17 kDa

Deubiquitinating Enzymes

Ubiquitin Specific Protease 25 His_6-tagged, *human recombinant*

E-546 50 µg

USP25 (Ubiquitin Specific Protease 25) is a deubiquitinating enzyme of the C19 family that is expressed in many human tissue types. USP25 activity is modulated by a sumoylation event that is dependent on the presence of a SUMO-interaction domain (SIM) located in the amino-terminal region of all USP25 isoforms. Sumoylation occurs within one of two ubiquitin interaction motifs (UIMs) that are required for efficient in vitro hydrolysis of polyubiquitin chains (K48-linked or K63-linked) by USP25. While sumoylation impairs USP25 activity again polyubiquitin substrates, it does not affect its hydrolysis of ubiquitin-AMC. This protein contains a C-terminal 6-His tag.

Purity: >90% by SDS-PAGE **MW:** 123 kDa

STAM-binding protein AMSH, *human recombinant*

E-548 25 µg

AMSH (STAM binding protein) is a zinc metalloprotease belonging to the JAMM (JAB1/MPN/Mov34) family of deubiquitinating enzymes (DUBs). This enzyme functions at the endosome, where it is involved in the sorting of various cell-surface receptors to lysozomes. AMSH cleaves K63-linked but not K48-linked polyubiquitin chains, and the in vitro activity of AMSH is greatly increased in the presence of STAM protein. This recombinant protein is full-length.

Purity: > 90% by SDS-PAGE **MW:** 48 kDa

Signal transducing adapter molecule 1, *human recombinant*

E-550 50 µg

STAM-1 (Signal Transducing Adapter Molecule 1) is involved in multiple signal transduction pathways through interactions mediated by ITAM, SH3, UIM, and VHS domains located within the protein. Roles in growth factor receptor down-regulation, T-cell development, and induction of DNA synthesis following IL-2 stimulation have been identified. This full-length recombinant protein has no intrinsic deubiquitinase activity, but is useful in stimulating the in vitro activity of the JAMM-class deubiquitinase AMSH.

Purity: > 90% by SDS-PAGE **MW:** 59 kDa

Deubiquitinase USP9x, isoform 2, His6-tagged, *human recombinant*

E-552 25µL

USP9x (homologue of drosophila Protein Fat Facets) is a deubiquitinating enzyme of the C19 peptidase family. USP9x is an essential component of TGFß signaling, where it functions at least partially through deubiquitination of SMAD4. USP9x may also generate important signaling events in pancreatic ductal adenocarcinoma and chronic myelogenous leukemia. This recombinant protein contains an N-terminal 6-His tag.

Purity: > 85% by SDS-PAGE **MW:** 292 kDa

Deubiquitinase Otubain 2, His6-tagged, *human recombinant*

E-554 50µL

Otubain-2 is a C65 type peptidase and a member of the ovarian tumor (OTU) protein super-family found in eukaryotes, viruses and pathogenic bacterium. Otubain-2 associates with the E3 ubiquitin ligases TRAF3 and TRAF6, and has been shown to negatively regulate virus-induced type I IFN induction and the antiviral responses mediated by these ligases. This recombinant protein contains a C-terminal 6-His tag.

Purity: >90% by SDS-PAGE **MW:** 28 kDa

Deubiquitinase CYLD, His6-tagged, *human recombinant*

E-556 50 µg

RNF4 (small nuclear ring finger protein, SNURF) is a RING-finger ubiquitin E3 ligase that ubiquitinates and mediates the proteasomal destruction of targets such as PML, PEA3, CENP1, and PARP1. In addition to the RING domain, RNF4 contains four SUMO-interacting motifs (SIMs) that function to recruit this ligase to poly-sumoylated substrates. RNF4 will autoubiquitinate in vitro, and will also ubiquitinate poly-SUMO chains. This recombinant protein is untagged.

Purity: > 95% by SDS-PAGE **MW:** 21 kDa

Deubiquitinase FAM105B/OTULIN, *human recombinant*

E-558 50 µg

FAM105B ("OTULIN") is a C65 type peptidase and a member of the ovarian tumor (OTU) protein super-family. OTULIN has exquisite specificity for linear (methionine-1-linked) ubiquitin linkages and has been shown to play a role in the regulation of NF-kB mediated signaling. This recombinant protein is untagged.

Purity: > 95% by SDS-PAGE **MW:** 41 kDa

Deubiquitinase ZRANB1/Trabid, *human recombinant*

E-560 50µg

Zinc Finger, RAN-binding Domain Containing 1 (ZRANB1), also known as TRAF-binding Domain-containing Protein (Trabid), is a C64 type peptidase and a member of the ovarian tumor (OTU) protein super-family with a predicted molecular weight of 81 kDa (1). The human protein shares 99% amino acid sequence identity with its mouse ortholog. ZRANB1 preferentially cleaves K29-, K33-, and K63-linked poly-Ubiquitin chains (2). It has been shown to play a role in the regulation of Wnt signaling via deubiquitination of APC (3,4). This recombinant protein contains a C-terminal 6-His tag.

Purity: > 95% by SDS-PAGE **MW:** 82 kDa

E2 Conjugating Enzymes

Ubiquitin conjugating enzyme UBE2G1, His_6-tagged, *human recombinant*

E2-700 50 µg
100 µg

UBE2G1 (E2-17K) is a homologue of the yeast Ubc7 protein. This E2 forms polyubiquitin chains in vitro, and may serve as an E2 for the CRL4Cdt2 ligase complex. This protein contains an N-terminal 6-His tag.

Purity: >95% by SDS-PAGE **MW:** 20.3 kD

Ubiquitin conjugating enzyme UBE2D4 His_6-tagged, *human recombinant*

E2-705 50 µg
100 µg

UBE2D (UbcH5) enzymes are human homologues of the yeast UBC4/5 family and play many important regulatory roles in inflammation and cancer. UBE2D4 (UbcH5D) has been reported to generate multiple ubiquitin chain linkage types in vitro, and may serve as the E2 carrier protein for numerous ubiquitin ligases. This protein contains N-terminal 6-His tag.

Purity: >95% by SDS-PAGE **MW:** 17.5 kD

Ubiquitin conjugating enzyme UBE2J2, isoform 1 His_6-tagged, *human recombinant*

E2-710 50 µg
100 µg

UBE2J2 is a transmembrane, endoplasmic reticulum-associated E2 that may be involved in targeting retrotranslocated, ER-associated proteins for proteasomal degradation (ERAD). This E2 is utilized by the γ-HV68 viral mK3 ligase in the polyubiquitination of substrates on serine, and/or threonine residues. The transmembrane and lumenal domains of this protein have been removed. This protein contains an N-terminal 6-His tag.

Purity: >95% by SDS-PAGE **MW:** 26.3 kD

Ubiquitin conjugating enzyme UBE2R2, His_6-tagged, *human recombinant*

E2-715 50 µg

UBE2R2 (CDC34B) is highly homologous to both UBE2R1, and the yeast CDC34 protein. UBE2R2 is phosphorylated by Casein Kinase 2 in vivo and in vitro, and may play a role in β-catenin stability. This E2 has been reported to form K48-isopeptide linkages in vitro. This protein contains an N-terminal 6-His tag.
100 µg

Purity:>95% by SDS-PAGE **MW:** 28.0 kD

Ubiquitin conjugating enzyme UBE2Q1, isoform 2, His_6-tagged, *human recombinant*

E2-720 50 µg
100µg

UBE2Q1 is the E2 enzyme encoded by NICE-5, one of the genes of the human epidermal differentiation complex (EDC). The protein forms thiolester complexes with ubiquitin in vitro, and contains an N-terminal 6-His tag.

Purity:> 90% by SDS-PAGE **MW:** 29.3 kD

Ubiquitin conjugating enzyme UBE2W, isoform 2, His_6-tagged, *human recombinant*

E2-725 50 µg
100µg

UBE2W (Ubc16) may play a role in Fanconi anemia tumor suppressor pathway by mono-ubiquitinating FANCD2 when in complex with the E3 ligase FANCL. This E2 has also been shown to catalyze the autoubiquitination of the BRCA1/BARD1 E3 complex, and to form K11 linked polyubiquitin chains in vitro. This protein contains an N-terminal 6-His tag.

Purity: >90% by SDS-PAGE **MW:** 19.5 kD

Ubiquitin conjugating enzyme UBE2V2 His_6-tagged, *human recombinant*

E2-730 50 µg
100µg

UBE2V2 (MMS2) is not an active E2 in the absence of UBE2N. The UBE2V2/UBE2N heterodimer catalyzes the synthesis of K63-linked polyubiquitin chains and may be an E2 for the CHFR E3 ligase. This protein forms polyubiquitin chains in vitro and contains an N-terminal 6-His tag.

Purity: > 95% by SDS-PAGE **MW:** 17.2 kD

Ubiquitin conjugating enzyme UBE2W, isoform 1 His_6-tagged, *human recombinant*

E2-740 50 µg
100µg

UBE2W (Ubc16) may play a role in Fanconi anemia tumor suppressor pathway by mono-ubiquitinating FANCD2 when in complex with the E3 ligase FANCL. This E2 has also been shown to catalyze the autoubiquitination of the BRCA1/BARD1 E3 complex, and to form K11 linked polyubiquitin chains in vitro. This protein contains an N-terminal 6-His tag.

Purity: > 90% by SDS-PAGE **MW:** 18.1 kD

Ubiquitin conjugating enzyme UBE2Q2, isoform 1 His_6-tagged, *human recombinant*

E2-745 50 µg
100µg

UBE2Q2 has implicated roles in cell-cycle regulation, apoptosis, and cancer, and has been reported to form K48-type linkages in some in vitro assays. This protein contains an N-terminal 6-His tag.

Purity: > 95% by SDS-PAGE **MW:** 43.6 kD

Ubiquitin conjugating enzyme UBE2J1, His_6-tagged, *human recombinant*

E2-750 50 µg
100µg

UBE2J1 is a human homologue of the yeast Ubc6 protein. In yeast, this tail-anchored transmembrane E2 is localized to the cytoplasmic surface of the ER and participates in ER-associated degradation (ERAD) of misfolded proteins. UBE2J1 is reported to play a role in degradation of misfolded MHC class I molecules via a mechanism requiring the E3 ubiquitin ligase Hrd-1. This protein contains an N-terminal 6-His tag, and the C-terminal transmembrane and lumenal amino acid sequences have been removed.

Purity: > 95% by SDS-PAGE **MW:** 32.0 kD

Ubiquitin conjugating enzyme UBE2Q1, isoform 1, His_6-tagged, *human recombinant*

E2-755 50 µg
100µg

UBE2Q1 is the E2 enzyme encoded by NICE-5, one of the genes of the human epidermal differentiation complex (EDC). The protein forms thioester complexes with ubiquitin in vitro, and contains an N-terminal 6-His tag.

Purity: >90% by SDS-PAGE **MW:** 46.9 kD

Ubiquitin-like Modifiers

Ubiquitin-like modifier FUBI/MNSFβ, *human recombinant*

UL-930 250 µg

"The Ubiquitin-like protein FUBI/MNSFß (Monoclonal Nonspecific Suppressor Factor Beta) is encoded by the FAU gene, and is translated as a pro-form consisting of FUBI fused to the ribosomal S30 protein. S30 is cleaved in a post-translational reaction, releasing the mature 74 amino acid FUBI/MNSFß protein. FUBI has a C-terminal gly-gly motif common to many ubiquitin-like proteins. FUBI/MNSFß conjugation to Bcl-G has been shown to regulate the ERK 1/2-MAPK cascade in macrophage cell lines, and may be implicated in TLR4-mediated signal transduction. Conjugation to endophilin II regulates dectin-1-mediated phagocytosis and inflammatory responses, and may be implicated in TLR2 signaling pathway."

Purity: > 95% by SDS-PAGE **MW:** 7.8 kDa

Ubiquitin-related modifier 1 protein, *human recombinant*

UL-950 250 µg

URM1 (Ubiquitin-related modifier 1) is a protein that functions in the post-translational modification of proteins via the Urmylation pathway. In humans, Urm1 is thiocarboxylated (-COSH) at its C-terminus by the MOCS3 protein in a two-step process that is highly reminiscent of ubiquitin activation by E1 enzymes. It's been suggested that Urm1 plays a role in oxidative-stress response in mammals, and that the protein may be an evolutionary link between ancient ubiquitin progenitors and the eukaryotic ubiquitin/ubiquitin-like modification systems. This protein is untagged.

Purity: >99% by SDS-PAGE **MW:** 11 kDa

Ubiquitin Mutants

Ubiquitin mutant L73P, *human recombinant*

UM-L73P 1 mg

This ubiquitin (Ub) contains a mutation of leucine 73 to proline. Ub L73P can form an E1-catalyzed active thioester at the C-terminus allowing the molecule to be transferred to E2 carrier proteins, and possibly to E3 ligases and their substrates. Polyubiquitin chains generated using L73P are highly resistant to disassembly by deubiquitinases. Potential uses of this protein include generating ubiquitin conjugates that are more stable than their native counterparts in the presence of deubiquitinase enzymes.

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

DCA-linked Non-hydrolyzable Chains

Di-Ubiquitin/Ub2, K6-DCA-linked *human recombinant*

UCD-10 100 µg

Dichloroacetone (DCA) linked forms of Ub2 are chemically linked via a DCA linker using cysteine introduced into the ubiquitin sequence at the appropriate residues to mimic a native isopeptide linkage. Reports have demonstrated that the DCA linkage is stable and cannot be processed by deubiquitinating enzymes. Additionally, the orientation of the two ubiquitins in DCA-linked Ub2 is preserved in relation to di-ubiquitin containing a native isopeptide linkage. This makes DCA-linked Ub2 useful for characterizing polyubiquitin-binding proteins in the absence of added deubiquitinase inhibitors.

Purity: > 95% by SDS-PAGE and HPLC **MW:** 17 kDa

Di-Ubiquitin/Ub2, 6-DCA-linked, Agarose, *human recombinant*

UCD-12 100 µL

Dichloroacetone (DCA) forms of di-ubiquitin (Ub2) are chemically linked via a DCA linker using cysteine introduced into the ubiquitin sequence at the appropriate residues to mimic a native isopeptide linkage. Reports have demonstrated that the DCA linkage is stable and cannot be processed by deubiquitinases. Additionally, the orientation of the two ubiquitins in DCA-linked Ub2 is preserved in relation to di-ubiquitin containing a native isopeptide linkage. This makes DCA-linked Ub2 useful for characterizing polyubiquitin-binding proteins in the absence of added deubiquitinase inhibitors.

Di-Ubiquitin/Ub2, 11-DCA-linked, *human recombinant*

UCD-40 100 µg

Dichloroacetone (DCA) forms of di-ubiquitin (Ub2) are chemically linked via a DCA linker using cysteine introduced into the ubiquitin sequence at the appropriate residues to mimic a native isopeptide linkage. Reports have demonstrated that the DCA linkage is stable and cannot be processed by deubiquitinases. Additionally, the orientation of the two ubiquitins in DCA-linked Ub2 is preserved in relation to di-ubiquitin containing a native isopeptide linkage. This makes DCA-linked Ub2 useful for characterizing polyubiquitin-binding proteins in the absence of added deubiquitinase inhibitors.

Purity: > 95% by SDS-PAGE and HPLC **MW:** 17 kDa

Di-Ubiquitin/Ub2, 11-DCA-linked, Agarose, *human recombinant*

UCD-42 100 µL

Dichloroacetone (DCA) forms of di-ubiquitin (Ub2) are chemically linked via a DCA linker using cysteine introduced into the ubiquitin sequence at the appropriate residues to mimic a native isopeptide linkage. Reports have demonstrated that the DCA linkage is stable and cannot be processed by deubiquitinases. Additionally, the orientation of the two ubiquitins in DCA-linked Ub2 is preserved in relation to di-ubiquitin containing a native isopeptide linkage. This makes DCA-linked Ub2 useful for characterizing polyubiquitin-binding proteins in the absence of added deubiquitinase inhibitors.

Di-Ubiquitin/Ub2, K29-DCA-linked *human recombinant*

UCD-80 100 µg

Dichloroacetone (DCA) forms of di-ubiquitin (Ub2) are chemically linked via a DCA linker using cysteine introduced into the ubiquitin sequence at the appropriate residues to mimic a native isopeptide linkage. Reports have demonstrated that the DCA linkage is stable and cannot be processed by deubiquitinases. Additionally, the orientation of the two ubiquitins in DCA-linked Ub2 is preserved in relation to di-ubiquitin containing a native isopeptide linkage. This makes DCA-linked Ub2 useful for characterizing polyubiquitin-binding proteins in the absence of added deubiquitinase inhibitors.

Purity: > 95% by SDS-PAGE and HPLC **MW:** 17 kDa

Di-Ubiquitin/Ub2, 29-DCA-linked, Agarose, *human recombinant*

UCD-82 100 µL

Dichloroacetone (DCA) forms of di-ubiquitin (Ub2) are chemically linked via a DCA linker using cysteine introduced into the ubiquitin sequence at the appropriate residues to mimic a native isopeptide linkage. Reports have demonstrated that the DCA linkage is stable and cannot be processed by deubiquitinases. Additionally, the orientation of the two ubiquitins in DCA-linked Ub2 is preserved in relation to di-ubiquitin containing a native isopeptide linkage. This makes DCA-linked Ub2 useful for characterizing polyubiquitin-binding proteins in the absence of added deubiquitinase inhibitors.

Di-Ubiquitin/Ub2, 76-DCA-linked, *human recombinant*

UCD-90 25 µg

Dichloroacetone (DCA) linked forms of Ub2 are chemically linked via a DCA linker using cysteine introduced into the ubiquitin sequence at the appropriate residues to mimic a native isopeptide linkage. Reports have demonstrated that the DCA linkage is stable and cannot be processed by deubiquitinating enzymes. Additionally, the orientation of the two ubiquitins in DCA-linked Ub2 is preserved in relation to di-ubiquitin containing a native isopeptide linkage. Although Ub2 76-76 is not a naturally occurring linkage, this di-ubiquitin may be a useful control in experiments using the other DCA-linked di-ubiquitins.

Purity: > 95% by SDS-PAGE and HPLC **MW:** 17 kDa

Di-Ubiquitin/Ub2, 76-DCA-linked, Agarose, *human recombinant*

UCD-92 100 µL

Dichloroacetone (DCA) forms of di-ubiquitin (Ub2) are chemically linked via a DCA linker using cysteine introduced into the ubiquitin sequence at the appropriate residues to mimic a native isopeptide linkage. Reports have demonstrated that the DCA linkage is stable and cannot be processed by deubiquitinases. Additionally, the orientation of the two ubiquitins in DCA-linked Ub2 is preserved in relation to di-ubiquitin containing a native isopeptide linkage. This makes DCA-linked Ub2 useful for characterizing polyubiquitin-binding proteins in the absence of added deubiquitinase inhibitors. Although Ub2 76-76 is not a naturally occurring linkage, this di-ubiquitin may be a useful control in experiments using the other DCA-linked di-ubiquitins.

Di-Ubiquitin/Ub2, 33-DCA-linked, *human recombinant*

UCD-100 100 µg

Dichloroacetone (DCA) forms of di-ubiquitin (Ub2) are chemically linked via a DCA linker using cysteine introduced into the ubiquitin sequence at the appropriate residues to mimic a native isopeptide linkage. Reports have demonstrated that the DCA linkage is stable and cannot be processed by deubiquitinases. Additionally, the orientation of the two ubiquitins in DCA-linked Ub2 is preserved in relation to di-ubiquitin containing a native isopeptide linkage. This makes DCA-linked Ub2 useful for characterizing polyubiquitin-binding proteins in the absence of added deubiquitinase inhibitors.

Purity: > 95% by SDS-PAGE and HPLC **MW:** 17 kDa

Di-Ubiquitin/Ub2, 33-DCA-linked, Agarose, *human recombinant*

UCD-102 100µL

Dichloroacetone (DCA) forms of di-ubiquitin (Ub2) are chemically linked via a DCA linker using cysteine introduced into the ubiquitin sequence at the appropriate residues to mimic a native isopeptide linkage. Reports have demonstrated that the DCA linkage is stable and cannot be processed by deubiquitinases. Additionally, the orientation of the two ubiquitins in DCA-linked Ub2 is preserved in relation to di-ubiquitin containing a native isopeptide linkage. This makes DCA-linked Ub2 useful for characterizing polyubiquitin-binding proteins in the absence of added deubiquitinase inhibitors.

Di-Ubiquitin/Ub2, 48-DCA-linked, *human recombinant*

UCD-200 100 µg

Dichloroacetone (DCA) forms of di-ubiquitin (Ub2) are chemically linked via a DCA linker using cysteine introduced into the ubiquitin sequence at the appropriate residues to mimic a native isopeptide linkage. Reports have demonstrated that the DCA linkage is stable and cannot be processed by deubiquitinases. Additionally, the orientation of the two ubiquitins in DCA-linked Ub2 is preserved in relation to di-ubiquitin containing a native isopeptide linkage. This makes DCA-linked Ub2 useful for characterizing polyubiquitin-binding proteins in the absence of added deubiquitinase inhibitors

Purity: > 95% by SDS-PAGE and HPLC **MW:** 17 kDa

Di-Ubiquitin/Ub2, 48-DCA-linked, Agarose, *human recombinant*

UCD-202 100µL

Dichloroacetone (DCA) forms of di-ubiquitin (Ub2) are chemically linked via a DCA linker using cysteine introduced into the ubiquitin sequence at the appropriate residues to mimic a native isopeptide linkage. Reports have demonstrated that the DCA linkage is stable and cannot be processed by deubiquitinases. Additionally, the orientation of the two ubiquitins in DCA-linked Ub2 is preserved in relation to di-ubiquitin containing a native isopeptide linkage. This makes DCA-linked Ub2 useful for characterizing polyubiquitin-binding proteins in the absence of added deubiquitinase inhibitors.

Di-Ubiquitin/Ub2, 63-DCA-linked, *human recombinant*

UCD-300 25µg

Dichloroacetone (DCA) linked forms of Ub2 are chemically linked via a DCA linker using cysteine introduced into the ubiquitin sequence at the appropriate residues to mimic a native isopeptide linkage. Reports have demonstrated that the DCA linkage is stable and cannot be processed by deubiquitinating enzymes. Additionally, the orientation of the two ubiquitins in DCA-linked Ub2 is preserved in relation to di-ubiquitin containing a native isopeptide linkage. This makes DCA-linked Ub2 useful for characterizing polyubiquitin-binding proteins in the absence of added deubiquitinase inhibitors.

Purity: > 95% by SDS-PAGE and HPLC **MW:** 17 kDa

Di-Ubiquitin/Ub2, 63-DCA-linked, Agarose, *human recombinant*

UCD-3021 100µL

Dichloroacetone (DCA) forms of di-ubiquitin (Ub2) are chemically linked via a DCA linker using cysteine introduced into the ubiquitin sequence at the appropriate residues to mimic a native isopeptide linkage. Reports have demonstrated that the DCA linkage is stable and cannot be processed by deubiquitinases. Additionally, the orientation of the two ubiquitins in DCA-linked Ub2 is preserved in relation to di-ubiquitin containing a native isopeptide linkage. This makes DCA-linked Ub2 useful for characterizing polyubiquitin-binding proteins in the absence of added deubiquitinase inhibitors.

SUMO Chains

Poly-SUMO2 Hydrolysis Resistant Chains (3-8)

ULN-220 50 µg

Sentrin protease (SENP)-resistant poly-SUMO2 chains can be used to investigate mechanisms of chain recognition by SUMO-targeted ubiquitin ligases (STUbl's) or other proteins that contain SUMO-interacting domains (SIM's). These K11-linked chains are formed enzymatically with recombinant SUMO2 containing a mutation of glutamine 90 to proline, which renders them approximately 500-fold more resistant to disassembly by SENP's than are wild-type chains. Mono- and di-SUMO2 have been removed from the chain mixture.

Purity: >90% by SDS-PAGE

Poly-SUMO3 Hydrolysis Resistant Chains (3-8), *human recombinant*

ULN-320 100 µg

Sentrin protease (SENP)-resistant poly-SUMO3 chains can be used to investigate mechanisms of chain recognition by SUMO-targeted ubiquitin ligases (STUbl's) or other proteins that contain SUMO-interacting domains (SIM's). These K-11 linked chains are formed enzymatically with recombinant SUMO3 containing a mutation of glutamine 89 to proline, which renders them approximately 500-fold more resistant to disassembly by SENP's than are wild-type chains. Mono- and di-SUMO3 have been removed from the chain mixture.

Purity: >90% by SDS-PAGE

Inhibitors

PS-341, 26S Proteasome Inhibitor Synthetic

I-200 5 mg

The dipeptide-boronic acid analogue PS-341 is the active ingredient in Bortezomib (Velcade, Millennium Pharmaceuticals). This potent, cell permeable molecule inhibits primarily the chymotryptic activity of the proteasome without substantially altering tryptic or caspase-like activities. PS-341 inhibits proliferation of a number of tumor cell lines with nanomolar potency, and is approved by the USFDA for the treatment of multiple myeloma.

Purity: >99% by HPLC and TLC **FW:** 384

Nedd8 E1 Activating Enzyme Inhibitor Synthetic

I-502 1 mg

NAE Inhibitor contains the active component of MLN4924, a cell-permeable, small molecule inhibitor of Nedd8 activating enzyme (NAE). By inhibiting the Neddylation pathway this molecule disrupts cullin-RING ligase activity, leading to inhibition of proliferation or apoptotic death of various human tumor cells through the deregulation of S-phase DNA synthesis. Mutations in UBA3, a gene encoding one of the subunits of Nedd8 activating enzyme, have been reported to cause resistance to MLN4924 in some tumor cell lines.

Purity: >99% by HPLC **FW:** 444

Buffers

10X E3 Ligase Reaction Buffer

B-71 5 mL

E3 Ligase Reaction Buffer is formulated to support the in vitro activity of E3 Ligase enzymes. This buffer is similar in composition to 10X Ubiquitin Conjugation Reaction Buffer (B-70), but contains a higher concentration of reductant for those enzymes that benefit from additional disulfide-reducing potential. Fully compatible with E1 and E2 enzyme activity, this buffer can be used to supplement the ubiquitin-protein conjugation kit (K-960), fraction II conjugation kits (K-930, K-935), and E3-ligase enzymes.

10X HSP Reaction Buffer

B-72 5 mL

HSP Reaction Buffer is formulated to support the in vitro enzymatic activity of Heat Shock Proteins. This buffer is similar in composition to 10X E3 Ligase Reaction Buffer (B-71), but contains potassium chloride that improves the catalytic function of HSP70 in vitro. Fully compatible with E1, E2, and E3 ubiquitin ligase enzyme activities, this buffer can be used for in vitro protein refolding experiments, or in ubiquitination reactions that require HSP activity. This buffer does not contain Mg2+-ATP.

Associated Proteins

HSP70/HSPA1A, *human recombinant*

AP-100 50 µg
100 µg

Heat shock proteins (HSPs) are a family of highly conserved stress response proteins. Heat shock proteins function primarily as molecular chaperones by facilitating the folding of other cellular proteins, preventing protein aggregation or targeting improperly folded proteins to specific degradative pathways. HSPs are typically expressed at low levels under normal physiological conditions, but are dramatically up-regulated in response to cellular stress. HSP70 is a 72 kDa member of the heat shock protein 70 family of proteins, and is also known as HSPA1A, HSP70-1, and HSP72. HSP70 and HSP40 are required to target some misfolded proteins to the Ubiquitin E3 ligase CHIP (Stub1) for subsequent ubiquitination.

Purity: > 95% by SDS-PAGE **MW:** 70 kDa

HSP40/DNAJB1, *human recombinant*

AP-110 50 µg

Heat shock protein 40 (HSP40, also known as DNAJB1) is the human homologue of the bacterial DnaJ heat shock protein. Heat shock proteins (HSPs) are a highly conserved family of stress response proteins. HSPs function primarily as molecular chaperones, facilitating the folding of other cellular proteins, preventing protein aggregation, or targeting improperly folded proteins to specific degradative pathways. Heat Shock Proteins are ubiquitously expressed in all organisms, and they are induced in response to various types of environmental stresses like heat, cold, and oxygen deprivation. HSP40 is a stress inducible chaperone that colocalizes with HSP70 and can bind unfolded proteins and prevent protein denaturation and aggregation. The conserved amino terminal J domain can interact with HSP70 and stimulate its ATPase activity. HSP40 and HSP70 are required to target some misfolded proteins to the Ubiquitin E3 ligase CHIP (Stub1) for subsequent ubiquitination. The protein contains a C-terminal 6-His tag.

Purity: > 95% by SDS-PAGE **MW:** 39 kDa

E6, Human Papilloma Virus type 16, *recombinant*

AP-120 25µg

E6 (Early protein 6) is a viral protein produced in cells infected with the Human Papillomavirus. E6 forms a complex with the host cell ubiquitin ligase E6AP (E6-Associated-Protein) generating a ligase activity that polyubiquitinates tumor suppressors p53 and p73 and targets them to the 26S proteasome for degradation. As a result DNA damage and chromosomal instabilities increase, often leading to cell proliferation and cancer. The E6/E6AP complex also targets other substrates for ubiquitination, such as TERT, BAK1, FADD, and pro-CASP8—none of which appear to be substrates for E6AP in the absence of E6. This protein is untagged, and contains six cysteine-to-serine substitutions at positions 23, 58, 87, 104, 118, and 147 (HPV type 16 sequence numbering).

Purity: > 90% by SDS-PAGE **MW:** 19 kDa

Substrate Proteins

His^{6}-FLAG-p53, *human recombinant*

SP-452 20 µg

Tumor suppressor protein p53, a nuclear transcription factor, plays an essential role in the regulation of the cell cycle and is frequently mutated or inactivated in many cancers. Numerous post-translational modifications modulate p53 activity including ubiquitination, phosphorylation, acetylation and methylation. The stability of p53 is regulated via the ubiquitin-proteasome pathway (UPP). MDM2 is an oncogenic ubiquitin E3 ligase that ubiquitinates p53, inhibits its transcriptional activity and promotes its degradation. Other E3 ligases that promote the proteasome-mediated degradation of p53 include Pirh2, COP1 and p300. USP7 (HAUSP) 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. This recombinant protein contains N-terminal 6-His and FLAG tags.

Purity: >75% by SDS-PAGE **MW:** 46 kDa