Lot # XXXXX

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MATERIAL DATA SHEET

Deconjugating Enzyme Set
Cat. # K-E10B

Attachment of ubiquitin or polyubiquitin to substrate proteins generates important biological signaling cues that are inherent to the linkage type of the polyubiquitin chain. For example, K48-linked polyubiquitin chains result in proteasome-mediated degradation of proteins to which they are attached, whereas K63-linked polyubiquitin chains play roles in various intracellular signaling cascades. An important feature of protein ubiquitination is that it is reversible. Substrate-anchored chains may be edited or removed from proteins by specialized proteases called deubiquitinating enzymes (DUBs). Deubiquitinase activity is often modulated by multiple parameters, including 1) specificity for a protein substrate(s) to which polyubiquitin chains are conjugated, 2) protein cofactor(s) that may be required for DUB activation, or 3) preference for polyubiquitin linkage-types.

In humans there are 80-90 identified DUBs that are divided into 5 broad categories based on domain structure and mechanism of action. These include the ubiquitin-specific protease (USP), ubiquitin C-terminal hydrolase (UCH), ovarian tumor-related protease or otubain (OTU), Machado-Joseph disease (MJD) and JAB1/MPN/Mov34 (JAMM) enzymes. The JAMM class enzymes are metalloproteases and utilize an active-site zinc, while the catalytic domains of the other four classes are based on a reactive cysteine residue contained within a catalytic triad. DUBs often contain ubiquitin-interaction motifs (UIMs) which bind to and recognize Ub/Ub chains helping to recruit poly-ubiquitinated substrates. UCHs have a preference for small substrates and their primary role is Ub recycling from C-terminal fusions such as lysine, glutathione, or peptide remnants. Large DUBs (such as USPs) may remove Ub from substrate proteins and disassemble polyubiquitin chains, thereby regulating protein-protein interactions and signaling pathways. Several DUBs function freely or in association with the 26S proteasome.

This kit contains the following deconjugating enzymes:

Isopeptidase T (USP5, IsoT), *human recombinant* - Isopeptidase T is a member of the high molecular weight ubiquitin C-terminal hydrolases (UCHs). This enzyme primarily disassembles free poly-ubiquitin chains linked through isopeptide bonds. Accession # NP_003472.

Ubiquitin C-terminal Hydrolase L3 (UCH-L3), *human recombinant*- UCH-L3 is a member of the low molecular weight UCHs that processes ubiquitin precursors and ubiquitinated proteins to generate monomeric ubiquitin. Accession # NP_005993.

Ubiquitin C-terminal Hydrolase L1 (UCH-L1), *human recombinant*- UCH-L1 is a member of the low molecular weight UCHs that processes ubiquitin precursors and ubiquitinated proteins to generate monomeric ubiquitin. Accession # NP_004172.

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His_6-Ataxin-3 (MJD protein 1), *human recombinant*- 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. (Accession # NP_004984).

His_6-$A20_{CD}$ (TNFAIP3 catalytic domain), *human recombinant* - A20 (TNFα-induced protein 3) is a cytoplasmic zinc finger protein that inhibits NFκB activity and tumor necrosis factor-mediated programmed cell death. A20 contains an N-terminal domain which has deubiquitinating enzyme activity and removes ubiquitin chains from receptor-interacting protein (RIP), thus mediating distinct regulatory effects in the down-regulation of NFκB signaling. Accession # NP_006281.

His_6-BAP1, *human recombinant*- BAP1 (BRCA1 Associated Protein 1) interacts with the RING finger domain of the E3 ligase BRCA1 (Breast Cancer1, early onset protein) which functions as a tumor suppressor in the BRCA1 growth control pathway. 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. Accession # NP_004647.

His_6-$USP2_{CD}$ (UBP41 catalytic domain), *human recombinant* - 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. Accession # NP_004196.

His_6-$USP7_{FL}$ (HAUSP), *human recombinant*- 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. Accession # NP_003461.

His_6-$USP8_{FL}$ (UBPY), *human recombinant*- USP8 (Ubiquitin Specific Protease 8) is a growth-regulated deubiquitinating enzyme (DUB) with a role in endosomal sorting of receptor tyrosine kinases (RTKs) such as EGFR *in vivo*. USP8 has DUB activity on Ub chains *in vitro* and does not discriminate between K48- and K63-linked isopeptide bonds.

His_6-Otubain1, *human recombinant* - Otubains belong to the ovarian tumor (OTU) protein super-family present in eukaryotes, viruses and pathogenic bacterium. Accession # NP_060140.

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Product Information

Supplied:

Protein	MW	Quantity	Stock Concentration	Final Concentration
1. Isopeptidase T	97 kDa	5µg	X mg/ml (X µM)	0.05-5 nM
2. UCH-L3	26 kDa	5µg	X mg/ml (X µM)	0.05-5 nM
3. UCH-L1	25 kDa	5µg	X mg/ml (X µM)	0.05-5 nM
4. His_6-Ataxin-3	42 kDa	5µg	X mg/ml (X µM)	1-5 µM
5. His_6-$A20_{CD}$	46 kDa	5µg	X mg/ml (X µM)	1-5 µM
6. His_6-BAP1	81 kDa	5µg	X mg/ml (X µM)	1-5 µM
7. His_6-$USP2_{CD}$	42 kDa	5µg	X mg/ml (X µM)	1-5 µM
8. His_6-$USP7_{FL}$	128 kDa	5µg	X mg/ml (X µM)	1-5 µM
9. His_6-$USP8_{FL}$	131 kDa	5µg	X mg/ml (X µM)	1-5 µM
10. His_6-Otubain1	33 kDa	5µg	X mg/ml (X µM)	1-5 µM

Purity: >90% by SDS-PAGE

Use: Final concentration provided is for use *in vitro* and depends on conditions and substrate.

Storage: Store at -80°C. Avoid multiple freeze/thaw cycles.

For Laboratory Research Use Only, Not For Use in Humans

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