

# **UBIQUITIN & UBL SIGNALING**



# Ubiquitin, Ubiquitin-like Proteins & Their Derivatives

Ubiquitin SUMO NEDD8 ISG15 FAT10 Ubiquitin & Ubl Mutants Ubiquitin & Ubl Terminal and Side Chain Derivatives Ubiquitin & Ubl Chains

#### **Ubiquitin & Ubl Reactive Antibodies**

Ubiquitin-reactive Antibodies K<sup>63</sup>-linkage-specific Ubiquitin-reactive Antibody Blocking Peptides for UbI-reactive Antibodies

#### **Ubiquitin Remnant Profiling**

Activating Enzymes Conjugating Enzymes Ligases Deconjugating Enzymes

### **Target/Substrate Proteins**

Activating Enzymes NF-κB and IKKα p53 SUMOylation Substrates NEDDylation Substrates

### **Detection & Isolation Kits & Components**

Ubiquitin & Ubl Agarose Conjugates Ubiquitin-binding Domains Detection, Isolation and Modification Kits

### **Proteasome & Related Complexes**

11S Activator 19S Regulator Proteasome 20S Complex Proteasome Inhibitors Proteasome Substrates Proteasome 26S Proteins & Kits COP9 Signalosome (CSN) Tripeptidyl Peptidase (TPPII) Autophagy



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# **OVERVIEW**

biguitinvlation of cellular proteins is a highly complex, temporally controlled, and tightly regulated process that targets thousands of cellular proteins in a specific manner. It is carried out by a modular cascade of enzymes with high specificity towards target proteins. Ubiquitinylation has emerged as a critically important posttranslational modification playing major roles in regulating a broad array of basic cellular processes, such as cell division, differentiation, signal transduction, trafficking, and protein quality control. It is thus not surprising that aberrations in the system have been implicated in the pathogenesis of many diseases, including certain malignancies, neurodegenerative disorders and pathologies of the inflammatory and immune response

Post-translational protein modification can be divided into two	C
fundamental types: that associated with the incorporation or removal	C
of a functional group and that associated with the introduction of a	r
functional protein (Table 1).	6

Since its discovery in 1975, it has been apparent that ubiquitin has a fundamental importance in cellular biochemistry. A small protein of only 76 amino acids and a molecular weight of ~8.6 kDa, ubiquitin is a widely distributed protein, and one which is very highly conserved across phylogeny. Ubiquitin forms the basis for one of the most important and complex protein post-translational modifications, signaling for many different cellular events and closely interlinking with other post-translational modifications, such as phosphorylation and acetylation.

Ubiquitin is the 'parent' of a family of ubiquitin-like proteins (Ubls),

Functional group/entity	Functional protein
Phosphate (-PO <sub>3</sub> H)	Ubiquitin
Acetyl (Ac-/CH <sub>3</sub> CO-)	SUMO-1, 2, 3
Methyl (Me-/CH <sub>3-</sub> )	NEDD8
Sulphate (-SO <sub>3</sub> H)	ISG15
Lipid	FAT10
Carbohydrate	Urm1

Table 1: Types of post-translational modifications

of which at least 20 members are currently identified. Ubls confer diverse functions on their target proteins. They are involved in a broad range of biological processes including: signal transduction, cell cycle, embryogenesis, cytoskeletal regulation, metabolism, stress response, homeostasis, DNA replication and mRNA processing. In addition to similarity in their modes of action and functionality, the ubiquitin superfold forms a structural component, almost identical to that of ubiquitin, that is shared amongst Ubl family members. This provides a stable scaffold on which different epitopes can mediate specific interactions with binding proteins and intramolecular domains. It would appear that a common ancestor based on this superfold has evolved to give various proteins that are involved in diverse activities within the cell.

The fate of the modified substrate protein will depend upon the exact nature and extent of the modification.



Ubiquitin cascade showing activation, conjugation, ligation, deconjugation, and recycling steps

# **Ubiquitin, Ubiquitin-like Proteins & their Derivatives**

### Ubiquitin

Ubiquitin is the founding member of a family of structurally conserved proteins, the ubiquitin-like proteins (Ubls), which include the members SUMO-1, -2, and -3, NEDD8, ISG15, FAT10, and others. A wide variety of ubiquitin and ubiquitin-like proteins and their derivatives is offered, facilitating careful exploration and dissection of the complex processes in which these proteins are involved.

Product Name	Product #	Utility	Size
Ubiquitin	BML-UW8795	Marking proteins for a variety of cellular activities	5 mg
Ubiquitin, (agarose immobilized)	BML-UW8630	Affinity purification of ubiquitin-binding proteins	0.5 mL
Ubiquitin (human), (recombinant) (His-tag)	BML-UW8610	Detection and purification of ubiquitinylated substrates	1 mg
Ubiquitin (human), (recombinant) (GST-tag)	BML-UW8620	Studying ubiquitinylation <i>in vitro</i> and detection and purification of ubiquitinylated substrates	1 mg
Ubiquitin (human), (recombinant)	BML-UW0280	Use in ubiquitinylation studies.	1 mg
Ubiquitin (human), (fluorescein labeled)	BML-UW1240	Use in ubiquitinylation studies.	100 µg
Ubiquitin+1, (recombinant) (His-tag)	BML-UW8790	Recombinant frame-shift extended protein	100 µg
Ubiquitin5+1, (recombinant) (His-tag)	BML-UW8855	Polyubiquitinylated Ub <sup>+1</sup>	25 µg
[D <sup>77</sup> ]Ubiquitin (human), (recombinant) (untagged)	BML-UW0345	Incapable of C-terminal isopeptide bond formation	1 mg
K <sup>6</sup> -only Ubiquitin (human), (recombinant) (untagged)	BML-UW0210	Production of poly-ubiquitin chains via Lys <sup>6</sup> only	1 mg
K <sup>11</sup> -only Ubiquitin (human), (recombinant) (untagged)	BML-UW0215	Production of poly-ubiquitin chains via Lys <sup>11</sup> only	1 mg
K <sup>27</sup> -only Ubiquitin (human), (recombinant) (untagged)	BML-UW0220	Production of poly-ubiquitin chains via Lys <sup>27</sup> only	1 mg
K <sup>29</sup> -only Ubiquitin (human), (recombinant) (untagged)	BML-UW0225	Production of poly-ubiquitin chains via Lys <sup>29</sup> only	1 mg
K <sup>33</sup> -only Ubiquitin (human), (recombinant) (untagged)	BML-UW0230	Production of poly-ubiquitin chains via Lys <sup>33</sup> only	1 mg
K48-only Ubiquitin (human), (recombinant) (untagged)	BML-UW0235	Production of poly-ubiquitin chains via Lys <sup>48</sup> only	1 mg
K63-only Ubiquitin (human), (recombinant) (untagged)	BML-UW0240	Production of poly-ubiquitin chains via Lys63 only	1 mg
Ubiquitin, (biotinylated)	BML-UW8705	Detection and purification of ubiquitinylated substrates	100 µg
Ubiquitin (bovine), (native) (methylated)	BML-UW8555	Incapable of forming poly-ubiquitin chains via lysine linkages	1 mg
[(N $^{\Sigma}$ -biotinyl)Lys <sup>6</sup> ]Ubiquitin	BML-UW8470	Detection and purification of ubiquitinylated substrates	100 µg
[(N $^{\Sigma}$ -biotinyl)Lys <sup>6</sup> , (N $^{\Sigma}$ -biotinyl)Lys <sup>48</sup> ]Ubiquitin	BML-UW8475	Detection and purification of ubiquitinylated substrates	100 µg
[(N $^{\Sigma}$ -biotinyl)Lys <sup>6</sup> , (N $^{\Sigma}$ -biotinyl)Lys <sup>63</sup> ]Ubiquitin	BML-UW8480	Detection and purification of ubiquitinylated substrates	100 µg
Ubiquitin aldehyde, (recombinant)	BML-UW8450	Inhibitor of deubiquitinylating enzymes (DUBs)	50 µg
Ubiquitin-AMC	BML-SE211	Fluorogenic substrate for deubiquitinylating enzymes (DUBs)	25 µg
Ubiquitin vinyl sulphone, (HA-tag)	BML-UW0155	Covalent inhibitors for detection and identification of deubiquitinylating enzymes (DUBs)	25 µg
Ubiquitin vinyl methyl ester, (HA-tag)	BML-UW0880	Covalent inhibitors for detection and identification of deubiquitinylating enzymes (DUBs)	25 µg
Ubiquitin-Rhodamine	BML-SE761	Substrate for deubiquitinylating assays	25 µg

For ubiquitin mutants, see page 7; and for ubiquitin chains, see page 10.

#### **SUMO**

Like ubiquitin, the SUMO proteins are protein modifiers that are covalently attached to the epsilon-amino groups of lysine residues within substrates and play an important role in a wide variety of biological processes. The mammalian SUMO family includes SUMO-1, -2, -3, and -4. All members are expressed in precursor forms and must be C-terminally processed to give the functionally active mature forms.

In contrast to ubiquitinylation, SUMO conjugation is highly specific in terms of target lysine residues, but many aspects of substrate and lysine selection by the SUMO-conjugating machinery still await clarification. SUMOylation events usually occur at a consensus motif, although not all such motifs are modified, demonstrating a need for additional specificity determinants in SUMOylation. In other cases, modification occurs at non-consensus sites. The regulation of SUMOylation is intimately linked to other post-translational modifications, including ubiquitinylation, phosphorylation and acetylation. While target proteins are predominantly conjugated to monomeric SUMO, all SUMO family members are able to form chains *in vitro*. In cells, SUMOs have the potential to polymerize via internal consensus sites for SUMOylation that are present in both SUMO-2 and SUMO-3. SUMO chain formation is reversible; SUMO polymers are disassembled by SUMO proteases both *in vitro* and *in vivo*. SUMO chains play roles in replication, in the turnover of SUMO targets by the proteasome and during mitosis and meiosis.<sup>[1]</sup>

There is a growing appreciation for the existence of cross-talk mechanisms between the SUMOylation and ubiquitinylation processes. Rather than being strictly parallel, these two systems have many points of intersection, and it is likely that the coordination of these two systems is a critical contributor to the regulation of many fundamental cellular events.

[1] SUMO chains: polymeric signals. A.C.Vertegaal; Biochem. Soc. Trans. 38, 46 (2010)

Product Name	Product #	Utility	Size
[K <sup>11</sup> R]SUM0-2 (human), (recombinant) (untagged)	BML-UW0515	Mono-/multi-SUMOylation of target proteins	100 µg
pro-SUMO-1 (human), (recombinant) (His-tag)	BML-UW9190	Regulation and processing studies	500 µg
pro-SUMO-2 (human), (recombinant) (His-tag)	BML-UW9200	Regulation and processing studies	500 µg
pro-SUMO-3 (human), (recombinant) (His-tag)	BML-UW9210	Regulation and processing studies	500 µg
SUM0-1 (93-97)-AMCA	BML-UW0500	Fluorogenic substrate for deSUMOylating enzymes	1 mg
SUMO-1 (human) (1-101), (recombinant)	ALX-201-044	Inactive precursor of human SUMO-1	250 µg
SUMO-1 (human) (1-97), (recombinant)	ALX-201-045	Protein conjugation studies	500 µg
SUMO-1 (human), (recombinant) (agarose immobilized)	BML-UW0095	Affinity purification of SUMO-1 interacting proteins	0.5 mL
SUMO-1 (human), (recombinant) (biotin conjugate)	BML-UW0545	Detection and purification of SUMOylated substrates	100 µg
SUMO-1 (human), (recombinant) (His-tag)	BML-UW9195	Functional studies	500 µg
SUMO-1-AMC	BML-UW0040	Fluorogenic substrate for deSUMOylating enzymes	50 µg
SUMO-2 (human) (1-93), (recombinant)	ALX-201-089	Conjugation to protein substrates	500 µg
SUMO-2 (human) (1-95), (recombinant)	ALX-201-088	Inactive precursor of human SUM0-2	250 µg
SUMO-2 (human), (recombinant) (agarose immobilized)	BML-UW0100	Affinity purification of SUMO-2 interacting proteins	0.5 mL
SUMO-2 (human), (recombinant) (His-tag)	BML-UW9205	Mature protein for functional studies	500 µg
SUMO-2 aldehyde	BML-UW0065	Specific inhibitor of deSUMOylating enzymes	25 µg
SUMO-2-AMC	BML-UW0045	Fluorogenic substrate for deSUMOylating enzymes	25 µg

Product Name	Product #	Utility	Size
SUMO-3 (human) (1-103), (recombinant)	ALX-201-086	Inactive precursor of human SUMO-3	250 µg
SUMO-3 (human) (1-92), (recombinant)	ALX-201-087	Conjugation to protein substrates	500 µg
SUMO-3 (human), (recombinant) (biotin conjugate)	BML-UW0555	Detection and purification of SUMOylated substrates	100 µg
SUMO-3 (human), (recombinant) (GST-tag)	BML-UW0170	Functional studies	500 µg
SUMO-3 (human), (recombinant) (His-tag)	BML-UW9215	Functional studies	500 µg
SUMO-3 (human), (recombinant) (agarose immobilized)	BML-UW0105	Affinity purification of SUMO-3 interacting proteins	0.5 mL
SUMO-4 (human), (recombinant) (His-tag)	BML-UW0905	Use in SUMO-4 conjugation studies	100 µg

#### SUMO Nomenclature

There is confusion within the scientific literature (including NCBI and UniProt protein databases) concerning the nomenclature used for SUMO-2 and SUMO-3 paralogs. Please note that Enzo Life Sciences uses the nomenclature proposed by Saitoh and Hinchey [J. Biol. Chem. 275, 6252 (2000)] for SUMO-2/SMT3A and SUMO-3/SMT3B and reports data accordingly.

### NEDD8

NEDD8 is a small ubiquitin-like protein that can be conjugated to substrate-proteins in a process known as NEDDylation. Although NEDDylation plays a critical regulatory role in cell growth, viability, and development, the spectrum of NEDD8 substrates and its interaction network remains the subject of much investigation. Originally believed to modify only the cullin family members, it is now recognized that a large number of NEDD8-modified and -associated proteins are involved in transcription, DNA repair and replication, cell cycle regulation and chromatin organization, and remodeling. Furthermore, mass spectrometric analyses has revealed that NEDD8 can form polymeric chains *in vivo*<sup>[2,3]</sup> with mechanisms for formation proposed<sup>[4]</sup>.

[2] A targeted proteomic analysis of the ubiquitin-like modifier nedd8 and associated proteins: J. Jones, et al.; J. Proteome Res. 7, 1274 (2008)
 [3] Novel substrates and functions for the ubiquitin-like molecule NEDD8: D.P. Xirodimas; Biochem. Soc. Trans. 36, 802 (2008)

[4] The mechanism of poly-NEDD8 chain formation *in vitro*: Y. Ohki, *et al.*; BBRC 381, 443 (2009)

Product Name	Product #	Utility	Size
NEDD8 (human), (recombinant) (agarose immobilized)	BML-UW0110	Affinity purification of NEDD8 interacting proteins	0.5 mL
NEDD8 (human), (recombinant) (biotin conjugate)	BML-UW0560	Detection and purification of NEDDylated substrates	100 µg
NEDD8 (human), (recombinant) (His-tag)	BML-UW9225	Functional studies	500 µg
NEDD8 aldehyde	BML-UW0070	Potent, specific and reversible inhibitor of deNEDDylating enzymes	50 µg
NEDD8-AMC	BML-UW0050	Fluorogenic substrate for deNEDDylating enzymes	25 µg
pro-NEDD8 (human), (recombinant) (GST-tag)	BML-UW8740	Regulation and processing studies	100 µg
pro-NEDD8 (human), (recombinant) (His-tag)	BML-UW9220	Regulation and processing studies	500 µg

### **ISG15**

A less appreciated and understood member of the ubiquitin-like protein family is ISG15, a modifier encoded by an interferon-stimulated gene. ISG15 has been ascribed important functions in various biological pathways from pregnancy to innate immune responses. Furthermore, ISG15 has been found to modify several important molecules and affect type I interferon signal transduction. Much further work is required in order to further elucidate the biological consequences of ISG15 and ISG15 modification<sup>[5]</sup>, although its role in certain disease states such as malignant transformation has recently been proposed<sup>[6]</sup>.

[5] ISG15: the immunological kin of ubiquitin: K.J. Ritchie & D.E. Zhang; Semin. Cell Dev. Biol. 15, 237 (2004)

[6] Expression, regulation and function of the ISGylation system in prostate cancer: A. Kiessling, et al.; Oncogene 28, 2606 (2009)

Product Name	Product #	Utility	Size
ISG15 (human), (recombinant) (agarose immobilized)	BML-UW0115	Affinity purification of ISG15 interacting proteins	0.5 mL
ISG15 (human), (recombinant) (His-tag)	BML-UW9235	Functional studies	500 µg
pro-ISG15 (human), (recombinant) (His-tag)	BML-UW9230	Regulation and processing studies	500 µg

#### FAT10

FAT10 is a small ubiquitin-like modifier that is encoded in the major histocompatibility complex and is synergistically inducible by tumor necrosis factor alpha and gamma-interferon. It is composed of two ubiquitin-like domains and possesses a free C-terminal diglycine motif that is required for the formation of FAT10 conjugates. FAT10 conjugates are rapidly degraded by the proteasome. Conjugation with FAT10 may thus provide an alternative ubiquitin-independent targeting mechanism for degradation by the proteasome, which is both cytokine-inducible and irreversible<sup>[7]</sup>. FAT10 has been shown to interact with the histone deacetylase HDAC6 which, in the absence of proteasomal degradation, may provide an alternative route to protein sequestration and removal by transporting conjugates to the aggresome<sup>[8]</sup>. Again, as with ISG15 modification, a role in malignant transformation has been proposed<sup>[9]</sup>.

[7] FAT10, a ubiquitin-independent signal for proteasomal degradation: M.S. Hipp, et al.; Mol. Cell Biol. 25, 3483 (2005).

[8] The ubiquitin-like modifier FAT10 interacts with HDAC6 and localizes to aggresomes under proteasome inhibition: B. Kalveram, et al.; J. Cell Sci. 121, 4079 (2008)

[9] FAT10 level in human gastric cancer and its relation with mutant p53 level, lymph node metastasis and TNM staging: F. Ji, et al.; World J. Gastroenterol. 15, 2228 (2009)

Product Name	Product #	Utility	Size
FAT10 (human), (recombinant) (His-tag)	BML-UW9240	Functional studies	250 µg
FAT10 (human), (recombinant) (agarose immobilized)	BML-UW0140	Affinity purification of FAT10 interacting proteins	0.5 mL

#### **Miscellaneous Ubls**

Product Name	Product #	Utility	Size
Fub1 (human), (recombinant) (His-tag)	BML-UW9535	Functional studies	100 µg
pro-Ubl5 (human), (recombinant) (His-tag)	BML-UW9495	Regulation and processing studies	100 µg
UbI5 (human), (recombinant) (His-tag)	BML-UW9525	Regulation and processing studies	100 µg
Urm1 (human), (recombinant) (His-tag)	BML-UW9530	Functional studies	100 µg

### [Lys/Arg]Ubiquitin and SUMO Mutants

[Lys/Arg]Ubiquitin and SUMO Mutants are useful for the production of poly-ubiquitin chains via specific lysine residues. The range consists of ubiquitin mutants containing only a single lysine at specific positions with all other lysines mutated to arginine, or ubiquitin mutants containing all but one lysine with the lysine concerned mutated to arginine. The mutation of lysine to arginine renders ubiquitin unable to form isopeptide linkages at that position. The ability to undergo thioester formation is preserved.

Product Name	Product #	Utility	Size
[E <sup>33</sup> R]SUMO-1 (human), (recombinant) (GST-tag)	BML-UW0175	Use in proteomic studies	100 µg
[K <sup>11</sup> R]SUMO-2 (human), (recombinant) (GST-tag)	BML-UW0380	Incapable of forming SUMO-2 chains at Lys <sup>11</sup>	100 µg
[K <sup>11</sup> R]SUMO-3 (human), (recombinant) (GST-tag)	BML-UW0385	Incapable of forming SUMO-3 chains at Lys <sup>11</sup>	100 µg
[K <sup>11</sup> R]SUMO-3 (human), (recombinant) (GST-tag)	BML-UW0520	Incapable of forming SUMO-3 chains at Lys <sup>11</sup>	100 µg
[K <sup>11</sup> R]Ubiquitin (human), (recombinant) (untagged)	BML-UW0250	Production of poly-ubiquitin chains via all lysines except Lys11	1 mg
[K <sup>27</sup> R]Ubiquitin (human), (recombinant) (untagged)	BML-UW0255	Production of poly-ubiquitin chains via all lysines except Lys <sup>27</sup>	1 mg
[K <sup>29</sup> R]Ubiquitin (human), (recombinant) (untagged)	BML-UW0260	Production of poly-ubiquitin chains via all lysines except Lys <sup>29</sup>	1 mg
[K <sup>33</sup> R]Ubiquitin (human), (recombinant) (untagged)	BML-UW0265	Production of poly-ubiquitin chains via all lysines except Lys <sup>33</sup>	1 mg
[K48R]Ubiquitin (human), (recombinant) (untagged)	BML-UW8615	Production of poly-ubiquitin chains via all lysines except Lys <sup>48</sup>	1 mg
[K <sup>63</sup> R]Ubiquitin (human), (recombinant) (untagged)	BML-UW0275	Production of poly-ubiquitin chains via all lysines except Lys <sup>63</sup>	1 mg
[K <sup>6</sup> R]Ubiquitin (human), (recombinant) (untagged)	BML-UW0245	Production of poly-ubiquitin chains via all lysines except Lys <sup>6</sup>	1 mg
[K <sup>all</sup> R]Ubiquitin (human), (recombinant) (untagged)	BML-UW0205	Negative control for poly-ubiquitinylation experiments	1 mg

### **Biotinylation**

Proteins are modified with biotin via reaction between a carboxyl group on biotin and primary amino groups within the protein being labeled. Depending upon the conditions used and subsequent purification procedures, this labelling results in multiple biotinylated species modified at the N<sup> $\alpha$ </sup>-amino group as well as on lysine N<sup> $\Sigma$ </sup>-amino groups. Although a fully functional C-terminus is maintained, lysine amino-group modification may limit the ability to propagate polyubiquitin chains. Biotinylated proteins can be detected using avidin-based enzyme reagents.

#### **Methylated Ubiquitin**

Methylated ubiquitin remains competent for activation, conjugation, and ligation to substrate proteins; however, it is not able to form ubiquitin chains as all amino groups are blocked by dimethylation. To ensure that all N<sup> $\alpha$ </sup>- or N<sup> $\Sigma$ </sup>-chain initiation is inhibited, it is absolutely essential that material of the highest integrity be used. The efficient octadimethylation of ubiquitin is hard to achieve. Enzo Life Sciences' product has been prepared and analyzed under stringent conditions in order to ensure the integrity of the material supplied.

Product Name	Product #	Utility	Size
Ubiquitin, [N <sup><math>\Sigma</math></sup> -biotinyl-Lys <sup>6</sup> ]	BML-UW8470	Detection and purification of ubiquitinylated substrates	100 µg
Ubiquitin, [N <sup><math>\Sigma</math></sup> -biotinyl-Lys <sup>6</sup> , N <sup><math>\Sigma</math></sup> -biotinyl-Lys <sup>48</sup> ]	BML-UW8475	Detection and purification of ubiquitinylated substrates	100 µg
Ubiquitin, [N <sup><math>\Sigma</math></sup> -biotinyl-Lys <sup>6</sup> , N <sup><math>\Sigma</math></sup> -biotinyl-Lys <sup>63</sup> ]	BML-UW8480	Detection and purification of ubiquitinylated substrates	100 µg
Ubiquitin, biotinylated (randomly)	BML-UW8705	Detection and purification of ubiquitinylated substrates	100 µg
Ubiquitin, methylated	BML-UW8555	Incapable of forming poly-ubiquitin chains via lysine linkages	1 mg
SUM0-1, biotinylated (randomly)	BML-UW0545	Detection and purification of SUMOylated substrates	100 µg
SUM0-3, biotinylated (randomly)	BML-UW0555	Detection and purification of SUMOylated substrates	100 µg
NEDD8, biotinylated (randomly)	BML-UW0560	Detection and purification of NEDDylated substrates	100 µg

### **Ubiquitin & Ubl C-terminal and Side Chain Derivatives**

Product Name	Product #	Utility	Size
Ubiquitin-AMC	BML-SE211	Fluorogenic substrate for deubiquitinylating enzymes (DUBs)	25 µg
Ubiquitin vinyl sulphone, HA-tagged (HA-Ub-VS)	BML-UW0155	Covalent inhibitors for detection and identification of deubiquitinylating enzymes (DUBs)	25 µg
Ubiquitin vinyl methyl ester, HA-tagged (HA-Ub-VME)	BML-UW0880	Covalent inhibitors for detection and identification of deubiquitinylating enzymes (DUBs)	25 µg

### **HA-Ubiquitin Vinyl Sulphone**

For the detection and identification of deubiquitinylating enzymes, HA-Ub-VS is a a DUB active site-directed probe that acts as a potent and irreversible inhibitor of DUBs through covalent modification of the active site and as a specific probe for enzymes with DUB activity. The HA peptide sequence (YPYDVPDYA), derived from the influenza hemagglutinin protein, facilitates sensitive identification or purification of HA-Ub-VS modified DUBs through recognition by HA-reactive antibodies and/or anti-HA-agarose.

**Figure 1:** DUB active site probe assay: Western blot showing reactions containing HA-Ub-VS only (lane 1), HA-Ub-VS + USP2cd (lane 2, Prod. No.BML-UW9850), and HA-Ub-VS + GSTUCHL1 (lane 3, Prod. No. BML-UW9305), HA-Ub-VS modified proteins detected using HA-reactive polyclonal antibody (Sigma - H6908) at 1:2000 dilution.

Product Name	Product #	Utility	Size
SUMO-2 aldehyde	BML-UW0065	Specific inhibitor of deSUMOylating enzymes	25 µg
SUMO-1-AMC	BML-UW0040	Fluorogenic substrate for deSUMOylating enzymes	50 µg
SUM0-1 [93-97]-AMCA	BML-UW0500	Fluorogenic substrate for deSUMOylating enzymes	1 mg
SUM0-2-AMC	BML-UW0045	Fluorogenic substrate for deSUMOylating enzymes	25 µg
NEDD8 aldehyde	BML-UW0070	Potent, specific and reversible inhibitor of deNEDDylating enzymes	50 µg
NEDD8-AMC	BML-UW0050	Fluorogenic substrate for deNEDDylating enzymes	25 µg



### **Ubiquitin Chains**

Ubiquitin chains are useful as standards for chain synthesis, recognition, breakdown studies, for deubiquitinylating enzyme assays, and for polyubiquitin binding studies. Amongst other applications, the novel single isopeptide linkage-based polyubiquitinylated substrate products may find great utility for the detailed study of deconjugating enzyme and ubiquitin binding domain specificities. They have already proven of considerable utility in assisting in the definition of the isopeptide-linkage specificity of an ubiquitin-reactive monoclonal antibody<sup>[10]</sup>.

[10] Analysis of nondegradative protein ubiquitylation with a monoclonal antibody specific for lysine-63-linked polyubiquitin: H. Wang, et al.; PNAS 105, 20197 (2008)

Product Name	Product #	Utility	Size
Deca-ubiquitin (linear)	BML-UW0815		100 µg
([K <sup>6</sup> only]Ub)n-ubiquitinylated substrate	BML-UW0615		25 µg
([K <sup>11</sup> only]Ub)n-ubiquitinylated substrate	BML-UW0620		25 µg
([K <sup>27</sup> only]Ub)n-ubiquitinylated substrate	BML-UW0625		25 µg
([K <sup>29</sup> only)]Ubn-ubiquitinylated substrate	BML-UW0630		25 µg
([K <sup>33</sup> only]Ub)n-ubiquitinylated substrate	BML-UW0635	Substrates for deubiquitinylating enzyme assays and polyubiquitin binding studies	25 µg
([K <sup>48</sup> only]Ub)n-ubiquitinylated substrate	BML-UW0640		25 µg
([K <sup>63</sup> only]Ub)n-ubiquitinylated substrate	BML-UW0645		25 µg
Di-ubiquitin (K <sup>48</sup> -linked)	BML-UW9800		100 µg
Di-ubiquitin (K <sup>63</sup> -linked)	BML-UW0730		50 µg
Di-ubiquitin (linear)	BML-UW0775		100 µg
Hepta-ubiquitin (linear)	BML-UW0800		100 µg
Hexa-ubiquitin (linear)	BML-UW0795		100 µg
Nona-ubiquitin (linear)	BML-UW0810		100 µg
Octa-ubiquitin (linear)	BML-UW0805		100 µg
Penta-ubiquitin (linear)	BML-UW0790		100 µg

Product Name	Product #	Utility	Size
Poly-ubiquitin chains (Ub <sub>2-16</sub> ) (K <sup>48</sup> -linked)	BML-UW0670		100 µg
Poly-ubiquitin chains (Ub <sub>2-7</sub> ) (K <sup>63</sup> -linked)	BML-UW9570		100 µg
Polyubiquitin chains (Ub2-7), (linear) (recombinant)	BML-UW1010	Substrates for deubiquitinylating enzyme assays and polyubiquitin binding studies	100 µg
Tetra-ubiquitin (K <sup>48</sup> -linked)	BML-UW8645		25 µg
Tetra-ubiquitin (linear)	BML-UW0785		100 µg
Tri-ubiquitin (linear)	BML-UW0780		100 µg
Ubn-ubiquitinylated substrate	BML-UW0610		25 µg
Undeca-ubiquitin (linear)	BML-UW0820		100 µg
Poly-SUM0-3 chains ([SUM0-3]2-7)	BML-UW9675		25 µg
Poly-SUM0-2 chains ([SUM0-2] <sub>2-7</sub> )	BML-UW9670		25 µg

# **Ubiquitin & Ubl Reactive Antibodies**

While there are a large number of antibodies available that are capable of recognizing ubiquitin or other members of the UbI family, there are few that are as well defined in the scientific literature as the monoclonal antibodies BML-PW8805 and BML-PW8810 [clones FK1 and FK2]. These antibodies are capable of recognizing mono- and/or polyubiguitinylated species and, when used in concert, are capable of discriminating between these modification types. The introduction of the K<sup>63</sup>-linkage specific monoclonal antibody BML-PW0600 [clone HWA4C4]<sup>[11]</sup> signalled the very first commercially available ubiguitin isopeptide-linkage specific reagent. Such immunological tools are of huge value in the determination of ubiquitinylation status in a variety of applications.

[11] Immunoreactivity to Lys63-linked polyubiquitin is a feature of neurodegeneration. S. Paine, et al.; Neurosci. Lett. 460, 205 (2009).

Product Name	Product #	Specificity	Application	Size
Diglycyl Lysine monoclonal antibody (GX41)	ADI-908-310	Species independent	IP, WB	100 µg
Mono- and polyubiquitinylated conjugates monoclonal antibody (FK2)	BML-PW8810	Species independent	IHC, WB	500 µg
Mono- and polyubiquitinylated conjugates monoclonal antibody (FK2) (ATTO 488 conjugate)	BML-PW1335	Species independent	ICC	25 µL
Mono- and polyubiquitinylated conjugates monoclonal antibody (FK2) (biotin conjugate)	BML-PW0755	Species independent	WB	25 µL
Mono- and polyubiquitinylated conjugates monoclonal antibody (FK2) (fluorescein labeled)	BML-PW1210	Species independent	ICC	25 µL
Mono- and polyubiquitinylated conjugates monoclonal antibody (FK2) (HRP conjugate)	BML-PW0150	Wide range of species	ELISA, IHC, WB	25 µg / 100 µg
Polyubiquitin (K <sup>63</sup> -linkage-specific) monoclonal antibody (clone HWA4C4) (HRP conjugate)	BML-PW0605	Wide range of species	WB	25 µg / 100 µg
Polyubiquitin (K <sup>63</sup> -linkage-specific) monoclonal antibody (HWA4C4)	BML-PW0600	Wide range of species	ELISA, IHC, ICC, WB	25 µg / 100 µg
Polyubiquitinylated conjugates monoclonal antibody (FK1)	BML-PW8805	Species independent	IHC, WB	500 µg
Ub+1 polyclonal antibody	BML-PW9780	Human	WB	25 µg / 100 µg

#### **Ubiguitin-reactive Antibodies**

Antibodies BML-PW8805 (clone FK1) and BML-PW8810 (clone FK2), are specific for ubiquitinprotein conjugates and show no reactivity with free ubiquitin under recommended conditions of use. Clone FK1 recognizes only polyubiquitinylated proteins and not monoubiquitinylated proteins or free ubiquitin, whilst clone FK2 recognizes both mono- and poly-ubiquitinylated species but not free ubiquitin. By using these antibodies in concert, the degree of protein ubiquitinylation may be determined.

Figure 2: Immunodetection of single lysine-linked polyubiquitin chains by western blotting following SDS-PAGE using [A] BML-PW8805 (clone FK1) and [B] BML-PW8810 (clone FK2).





Product Name	Product #	Specificity	Application	Size
Ubiquitin monoclonal antibody (EX-9)	BML-PW0580	Species independent	WB	25 μL
Ubiquitin monoclonal antibody (P4D1)	BML-PW0930	Species independent	IHC, IP, WB	100 µg
Ubiquitin monoclonal antibody (P4D1) (HRP conjugate)	BML-PW0935	Species independent	WB	25 μL
Ubiquitin monoclonal antibody (P4G7)	ENZ-ABS142	Species independent	ELISA, WB	200 µL
Ubiquitin polyclonal antibody	ADI-SPA-200	Wide range of species	ICC, IHC (PS), WB	50 µg
Ubiquitin polyclonal antibody (DyLight™ 488 conjugate)	ADI-SPA-200-488	Wide range of species	FC	50 µg
Ubiquitin-protein conjugates polyclonal antibody (fluorescein labeled)	BML-PW1235	Species independent	ICC	25 µL

### K63-linkage-specific Ubiquitin-reactive Antibody

Modification of proteins by addition of K<sup>63</sup>-linked polyubiquitin chains is implicated in a variety of cellular events, including DNA repair, signal transduction and receptor endocytosis. BML-PW0600 specifically recognizes K<sup>63</sup>-linked polyubiquitin, but NOT any other isopeptide-linked (K<sup>6</sup>, K<sup>11</sup>, K<sup>27</sup>, K<sup>29</sup>, K<sup>33</sup>, or K<sup>48</sup>) polyubiquitinylated species. This unique monoclonal antibody is a powerful tool facilitating the analysis of K<sup>63</sup>-linked polyubiquitinylation.



Figure 3: Western blot following SDS-PAGE of single lysine mutant chains probed with pan-reactive mAb FK1 [A] (BML-PW8805) & K<sup>63</sup>-linkage specific mAb HWA4C4 [B] (BML-PW0600).



Figure 4: [A] Sections through the hippocampus in Alzheimer's Disease (AD), stained using mAb HWA4C4 (BML-PW0600) and showing differential staining of neurofibrillary tangles (NFTs). [B] Immunogold labelling TEM in AD using HWA4C4 mAb (BML-PW0600) provides evidence that K<sup>63</sup>-linked polyubiquitin is present in NFTs <sup>[11]</sup>.

Product Name	Product #	Specificity	Application	Size
FAT10 (human) monoclonal antibody (4F1)	BML-PW0765	Human	ICC, IP, WB	25 μL / 100 μL
FAT10 polyclonal antibody	BML-PW9680	Human, Mouse	IP, WB	25 μL / 100 μL
FAT10 polyclonal antibody	BML-PW9585	Human, Mouse	IP, WB	25 μL / 100 μL
Fub1 (human) polyclonal antibody	BML-PW9615	Human	WB	25 μL / 100 μL
ISG15 (human) polyclonal antibody	BML-PW9575	Human	WB	25 μL / 100 μL
NEDD8 (human) polyclonal antibody	BML-PW9340	Human	IP, WB	25 μL / 100 μL
SUMO-1 (human) (CT) polyclonal antibody	BML-PW9460	Human	WB	25 μL / 100 μL
SUMO-1 (human) (NT) polyclonal antibody	BML-PW8330	Human	IP, WB	25 μL / 100 μL
SUMO-1 (human) polyclonal antibody	BML-PW0505A	Human	ICC, IP, WB	25 μL / 100 μL
SUMO-2/3 (human) (NT) polyclonal antibody	BML-PW9465	Human	WB	25 μL / 100 μL
SUMO-2 (human) polyclonal antibody	BML-PW0510A	Human	ICC, IP, WB	25 μL / 100 μL
UbI5 (human) polyclonal antibody	BML-PW9605	Human	WB	25 μL / 100 μL
Urm1 (human) polyclonal antibody	BML-PW9595	Human	IHC, WB	25 μL

## **Blocking Peptides for Ubl Reactive Antibodies**

Product Name	Product #	uct # Utility	
Blocking peptide for ISG15 (human) polyclonal antibody (Prod. No. BML-PW9575)	BML-PP9590 Control peptide useful for demonstrating the specificity of the ISG15 polyclonal antibody (BML-PW9575)		100 µg
Blocking peptide for SUMO-1 (human) (CT) polyclonal antibody (Prod. No. BML-PW9460)	BML-PP9475	Control peptide useful for demonstrating the specificity of the SUMO-1 (C-terminal) polyclonal antibody (BML- PW9460)	100 µg
Blocking peptide for SUMO-1 (human) (NT) polyclonal antibody (Prod. No. BML-PW8330)	BML-PP9470	Control peptide useful for demonstrating the specificity of the SUMO-1 (N-terminal) polyclonal antibody (BML- PW8330)	100 µg
Blocking peptide for SUMO-2/3 (human) (NT) polyclonal antibody (Prod. No. BML-PW9465)	BML-PP9480	Control peptide useful for demonstrating the specificity of the SUMO-2/3 (N-terminal) polyclonal antibody (BML- PW9465)	100 µg

# **Ubiquitin Remnant Profiling**

The conjugation of ubiquitin and Ubls to substrates usually involves three steps: (i) an initial activation step catalyzed by a specific activating enzyme (E1) in which the C-terminus of the protein is activated for subsequent reaction; (ii) an intermediate step involving transfer of the protein from the E1 to a covalent linkage with a conjugating enzyme (E2); and (iii) in which the protein is transferred to an amino group on the substrate protein, usually facilitated by a ligase enzyme (E3). The E2/E3 interaction determines the target of the protein, dictating its specific biological function. The availability of high purity/high activity recombinant enzymes allows *in vitro* reconstitution of many of these pathway steps.

The complexity of the ubiquitin and ubiquitin-like protein cascades is considerable. In mammals, there are some ten activating enzymes known, some twenty plus conjugating enzymes, over eight hundred ligases, and almost one hundred deconjugating enzymes. These varied components work in a hierarchical context and, for appropriate modification with ubiquitin or a Ubl to occur, the correct combination of E1, E2, E3, substrate, and deconjugating enzyme must all work in concert. The cascades for the ubiquitin-like proteins appear not to be as complex as that of ubiquitin with a reduced number of component possibilities. The availability of high purity/high activity recombinant enzymes allows *in vitro* reconstitution of many of these pathway steps.

### Activating Enzymes (E1s) – Proteins

Product Name	Product #	Utility	Size
Ubiquitin activating enzyme E1 (human), (recombinant) (His-tag)	BML-UW9410	Ubiquitin-specific activation	50 µg
SUMO activating enzyme E1 (human), (recombinant)	BML-UW9330	SUMO-specific activation	25 µg
ISG15 activating enzyme (human), (recombinant) (His-tag)	BML-UW9955	ISG15-specific activation	25 µg
NEDD8 activating enzyme E1 (human), (recombinant) (His-tag)	ENZ-PRT112	NEDD8 conjugation reactions and NEDDylation pathway studies	50 µg

### Activating Enzymes (E1s) – Antibodies

Product Name	Product #	Specificity	Application	Size
SUMO-1 activating enzyme subunit SAE1 (human) poly- clonal antibody	ALX-210-328	Human	ICC, WB	50 µg
UBA6 (human) polyclonal antibody	BML-PW0525	Human	WB	25 μL / 100 μL
UBE1L (human) polyclonal antibody	ALX-210-391	Human	IHC (FS, PS), WB	100 µL
Ubiquitin activating enzyme (CT) polyclonal antibody	BML-PW8395	Wide range of species	WB	25 µL
Ubiquitin activating enzyme (NT) polyclonal antibody	BML-PW8385	Human, Rat, Mouse, Rabbit, Chicken, Cow	IHC, IP, WB	25 μL / 100 μL
Ubiquitin activating enzyme polyclonal antibody	BML-PW8390	Human, Rabbit, Chicken, Cow	IHC, IP, WB	25 μL / 100 μL

# Conjugating Enzymes (E2s) – Proteins

Product Name	Product #	Size
UBIQUITIN		
[C <sup>85</sup> A]UbcH5a (human), (recombinant) (His-tag)	BML-UW9055	100 µg
[C <sup>85</sup> A]UbcH5c (human), (recombinant) (His-tag)	BML-UW9075	100 µg / 1 mg
hHR6A (human), (recombinant) (His-tag)	BML-UW9635	100 µg
hHR6B (human), (recombinant) (His-tag)	BML-UW9640	100 µg
Uba6 (human), (recombinant)	BML-UW0350	50 µg
Ubc9 (human), (recombinant)	ALX-201-046	50 µg
Ubc9 (human), (recombinant) (untagged)	BML-UW9320	100 µg
UbcH1 (human), (recombinant) (GST-tag)	BML-UW9730	100 µg
UbcH1 (human), (recombinant) (untagged)	BML-UW9735	100 µg
UbcH10 (human), (recombinant) (untagged)	BML-UW0960	100 µg
UbcH12 (human), (recombinant) (His-tag)	BML-UW9145	100 µg
UbcH13/Mms2 (human), (recombinant) (His-tag)	BML-UW9565	100 µg
UbcH2 (human), (recombinant) (His-tag)	BML-UW9025	100 µg
UbcH3 (human), (recombinant) (His-tag)	BML-UW8730	100 µg
UbcH5a (human), (recombinant) (His-tag)	BML-UW9050	100 µg
UbcH6 (human), (recombinant) (His-tag)	BML-UW8710	100 µg
UbcH7 (human), (recombinant) (His-tag)	BML-UW9080	100 µg
UbcH8 (human), (recombinant) (His-tag)	BML-UW9135	100 µg
Ubiquitin-conjugating enzyme sampler pack	BML-UW8975	1 Pack
SUMO		
UbcH9	BML-UW9320	100 µg
NEDD8		
UbcH12 (human), (recombinant) (His-tag)	BML-UW9145	100 µg
Ube2F (human), (recombinant) (His-tag)	BML-UW0940	50 µg
ISG15		
UbcH8 (human), (recombinant) (His-tag)	BML-UW9135	100 µg

## Conjugating Enzymes (E2s) – Antibodies

Product Name	Product #	Specificity	Application	Size
UBA6 (human) polyclonal antibody	BML-PW0525	Human	WB	25 µL / 100 µL
Ubc9 polyclonal antibody	ALX-210-233	Human	ICC, WB	50 µg
Use1 (human) polyclonal antibody	BML-PW0770	Human	WB	25 μL / 100 μL

## Conjugating Enzymes (E2s) – Regulators

Product Name	Product #	Utility	Size
NSC697923	ENZ-CHM143	Cell-permeable and selective inhibitor of the ubiquitin- conjugating (E2) enzyme Ubc13-Uev1A	5 mg

### Ligases (E3s) – Proteins

Product Name	Product #	Size
UBIQUITIN		
Hdm2 (catalytic RING domain) (human), (recombinant) (GST-tag)	BML-UW0200	25 µg
MuRF1 (rat), (recombinant) (GST-tag)	BML-UW0405	25 µg
Rbx1 (human), (recombinant) (His-tag)	BML-UW0395	25 µg
SUMO		
PIAS1 (human), (recombinant) (GST-tag)	BML-UW9960	25 µg
RanBP2∆FG (human), (recombinant) (GST-tag)	BML-UW9455	100 µg

# Ligases (E3s) – Antibodies

Product Name	Product #	Specificity	Application	Size
AtCul3 polyclonal antibody	BML-PW0470	Arabidopsis	IP, WB	25 µL
AtRbx1 polyclonal antibody	BML-PW0465	Arabidopsis	WB	25 µL
CHIP polyclonal antibody	ENZ-ABS273	Human, Mouse	WB	100 µg
COP1 polyclonal antibody	BML-PW9725	Human, Mouse, Rat, Hamster, Monkey	IP, WB	25 μL/ 100 μL
Cullin 1 monoclonal antibody (AS97.1)	ADI-KAM-CC135	Human, Mouse, Rat, Bovine, Monkey, Rabbit	WB	50 µg / 200 µg
DDA1 polyclonal antibody	BML-PW0455	Human, Mouse	IP, WB	25 μL/ 100 μL
DDB1 polyclonal antibody	BML-PW0460	Human, Mouse	WB	25 µL
E6AP (human) monoclonal antibody (EX-8)	BML-PW0535	Human	WB	25 μL/ 100 μL
Herc5 (human) polyclonal antibody	BML-PW0920	Human	WB	25 μL/ 100 μL
Huwe1 (mouse) polyclonal antibody	BML-PW0950	Human, Mouse, Rat	WB	25 μL/ 100 μL
Parkin (human) polyclonal antibody	BML-PW9365	Human	IHC, IP, WB	25 μL/ 100 μL
PGP9.5 polyclonal antibody	ADI-905-520	Human	IHC, WB	1 mL

### **Deconjugating Enzymes (DCEs) – Proteins**

Deconjugating enzymes (DCEs) can hydrolyze a peptide, amide, ester or thioester bond at the C-terminus of ubiquitin, including the post-translationally formed isopeptide bonds found in mono-, multi-, and polyubiquitinylated conjugates. DCEs thus have the potential to regulate any ubiquitin/ Ubl-mediated cellular process. Their conservation and widespread occurrence in eukaryotes, prokaryotes and viruses shows that these proteases constitute an essential class of enzymes.

Mammals contain some 80-90 deubiquitinylating enzymes (DUBs) falling into five subfamilies, namely the ubiquitin C-terminal hydrolases (UCHs); the ubiquitin-specific peptidases (USPs); the ovarian tumor (OTU) domain proteins; the Josephin or Machado-Joseph disease (MJD) proteins, and the JAMM (Jab1/MPN domain-associated metalloisopeptidase) domain proteases. Most DUBs contain a catalytic domain that has sequence similarity within subfamilies and structural similarity across subfamilies, and unrelated sequences either N-terminal or C-terminal (or both) to the catalytic domain. These flanking sequences have been shown to mediate substrate binding and presumably serve as substrate binding domains in all DUBs. They, along with the catalytic core, could also contribute to the binding and cleavage specificity for different ubiquitin-ubiquitin isopeptide linkages<sup>[12]</sup>.

Since most DUBs have been identified only by means of sequence similarity to catalytic motifs, there is little known functional information on many of these enzymes, with only a handful of these DUBs having been characterized with respect to the proteins with which they interact and deubiquitinylate. However, it is becoming increasingly apparent that DUBs must acquire their substrates by binding the target protein or by associating with other macromolecular complexes. Further study may reveal a variety of protein partners including substrates, scaffolds, adaptors and ubiquitin receptors. Much of the regulation and specificity of deubiquitinylation arises from the association of DUBs with these protein partners<sup>[13]</sup>.

The relatively few deconjugating enzymes characterized in detail to date provide insights into the crucial regulatory roles that they may play, making them potential drug target candidates for therapeutic intervention in ubiquitin/UbI-related diseases.

[12] Deubiquitylating enzymes and disease: S. Singhal, et al.; BMC Biochem. 9 Suppl. 1, S3 (2008)
[13] Protein partners of deubiquitinating enzymes: K.H. Ventii & K.D. Wilkinson; Biochem. J. 414, 161 (2008)

Product Name	Product #	Utility	Size
BAP1 (human), (recombinant) (His-tag)	BML-UW9855		50 µg
Isopeptidase T (long form) (human), (recombinant)	BML-UW9690		100 µg
Isopeptidase T (short form) (human), (recombinant)	BML-UW9695		25 µg
USP14 (human), (recombinant) (untagged)	BML-UW9840	Ubiquitin-specific proteases	25 µg
USP15 (human), (recombinant) (His-tag)	BML-UW9845		100 µg
USP2 (catalytic domain) (rat), (recombinant) (untagged)	BML-UW9850		50 µg
USP25 (isoform 2) (human), (recombinant) (His-tag)	BML-UW0475		100 µg
A20 (catalytic domain) (human), (recombinant) (untagged)	BML-UW1015		100 µg
NEDP1 (human), (recombinant)	ALX-201-171		50 µg
NEDP1 (human), (recombinant) (His-tag)	BML-UW9770	NEDD8-specific proteases	100 µg
SENP2 (catalytic domain) (human), (recombinant) (GST-tag)	BML-UW9765		100 µg
Otubain-1 (human), (recombinant) (His-tag)	BML-UW0680		50 µg
SENP1 (catalytic domain) (human), (recombinant) (GST-tag)	BML-UW9760	SUMU-specific proteases	100 µg
UCH-L1 (human), (recombinant) (His-tag)	BML-UW9740	Ubiquitin C-terminal hydrolase	50 µg
UCH-L3 (human), (recombinant) (His-tag)	BML-UW9745	BRCA1-associated ubiquitin C-terminal hydrolase	50 µg

# Deconjugating Enzymes (DCEs) – Substrates & Inhibitors

Product Name	Product #	Utility	Size
UBIQUITIN			
BML-282	BML-EI400	UCH-L1 inhibitor	5 mg
Hypothemycin	ALX-380-116	Ubiquitinylation enhancer of cyclin D1	250 µg / 1 mg
([K <sup>11</sup> only]Ub)n-ubiquitinylated substrate	BML-UW0620		25 µg
([K <sup>27</sup> only]Ub)n-ubiquitinylated substrate	BML-UW0625		25 µg
([K <sup>29</sup> only)]Ubn-ubiquitinylated substrate	BML-UW0630		25 µg
([K <sup>33</sup> only]Ub)n-ubiquitinylated substrate	BML-UW0635		25 µg
([K <sup>48</sup> only]Ub)n-ubiquitinylated substrate	BML-UW0640		25 µg
([K <sup>6</sup> only]Ub)n-ubiquitinylated substrate	BML-UW0615		25 µg
([K <sup>63</sup> only]Ub)n-ubiquitinylated substrate	BML-UW0645	Substrates for deubiquitinvlating enzyme assays and	25 µg
Di-ubiquitin (K <sup>48</sup> -linked)	BML-UW9800	polyubiquitin binding studies	100 µg
Di-ubiquitin (K <sup>63</sup> -linked)	BML-UW0730		50 µg
Linear polyubiquitin chains, sampler pack	BML-UW0825		1 Pack
Poly-ubiquitin chains (Ub <sub>2-16</sub> ) (K <sup>48</sup> -linked)	BML-UW0670		100 µg
Poly-ubiquitin chains (Ub <sub>2-7</sub> ) (K <sup>63</sup> -linked)	BML-UW9570		100 µg
Tetra-ubiquitin (K <sup>48</sup> -linked)	BML-UW8645		25 µg
Ubn-ubiquitinylated substrate	BML-UW0610		25 µg
TCID	BML-EI399	UCH-L3 inhibitor	10 mg / 50 mg
Ubiquitin-AMC	BML-SE211	Fluorogenic substrate for deubiquitinylating enzymes (DUBs)	25 µg
Ubiquitin aldehyde, (recombinant)	BML-UW8450	Inhibitor of deubiquitinylating enzymes (DUBs)	50 µg
Ubiquitin <sup>5+1</sup> , (recombinant) (His-tag)	BML-UW8855	Polyubiquitinylated Ub <sup>+1</sup>	25 µg
Ubiquitin vinyl methyl ester, (HA-tag)	BML-UW0880	Covalent inhibitors for detection and identification of	25 µg
Ubiquitin vinyl sulphone, (HA-tag)	BML-UW0155	deubiquitinylating enzymes (DUBs)	25 µg
Z-Leu-Arg-Gly-Gly-AMC	BML-P801		5 mg
Z-Arg-Leu-Arg-Gly-Gly-AMC	BML-ZW8585	Fluorogenic substrates for deubiquitinylating enzymes (DOBs)	5 mg
SUMO			
SUMO-2 aldehyde	BML-UW0065	Specific inhibitors of deSUMOylating enzymes	25 µg
SUMO-1-AMC	BML-UW0040		50 µg
SUMO-1 (93-97)-AMCA	BML-UW0500	Fluorogenic substrates for deSUMOylating enzymes	1 mg
SUMO-2-AMC	BML-UW0045		25 µg
Anacardic acid	ALX-270-381	Inhibitor of SUMOylation	5 mg / 25 mg
NEDD8	·		·
NEDD8 aldehyde	BML-UW0070	Potent, specific and reversible inhibitor of deNEDDylating enzymes	50 µg
NEDD8-AMC	BML-UW0050	Fluorogenic substrate for deNEDDylating enzymes	25 µg

### **Deconjugating Enzymes (DCEs) – Antibodies**

Product Name	Product #	Specificity	Application	Size
A20 monoclonal antibody (59A426)	ADI-AAM-009	Human	IHC, IP, WB	100 µg
AMSH (human) polyclonal antibody	BML-PW0655	Human	WB	25 μL / 100 μL
CYLD (human) polyclonal antibody	BML-PW0760	Human	ICC, WB	25 μL / 100 μL
MYSM1 (human) polyclonal antibody	BML-PW0660	Human	WB	25 μL / 100 μL
SENP2 (mouse) polyclonal antibody	ALX-210-482	Mouse	ELISA, WB	100 µg
SENP2 polyclonal antibody	ALX-210-863	Human, Mouse	WB	200 µL
SENP5 (human) polyclonal antibody	BML-PW0365	Human	WB	25 μL / 100 μL
SENP5 polyclonal antibody	ALX-210-865	Human, Mouse	WB	200 µL
SENP6 (human) polyclonal antibody	BML-PW0370	Human	WB	25 μL / 100 μL
USP15 (human) polyclonal antibody	BML-PW9795	Human, Mouse	WB	25 μL / 100 μL
USP18 (human) polyclonal antibody	BML-PW0945	Human	WB	25 μL / 100 μL
USP19 polyclonal antibody	BML-PW1025	Human, Mouse, Rat	WB	25 μL / 100 μL
USP21 (human) polyclonal antibody	BML-PW0585	Human	WB	25 μL / 100 μL
USP30 (human) polyclonal antibody	BML-PW0975	Human	WB	25 μL / 100 μL
USP7 (human) polyclonal antibody	BML-PW0540	Human	WB	25 μL / 100 μL

### UCH-L1 (PGP9.5), rabbit pAb

PGP9.5 (protein gene product 9.5) is abundant in many tissues, but especially so in neurons where it has been effectively used as a phenotypic marker<sup>[14-16]</sup>. PGP9.5 is a member of the ubiquitin C-terminal hydrolase family<sup>[17,18]</sup> and immunohistochemical studies have shown that the protein is enriched in several ubiquitinylated inclusion bodies, suggesting that such structures may be metabolically dynamic regions of the cell<sup>[19]</sup>. The antiserum may be used in Western blotting (24 kDa)<sup>[20]</sup> and has been used on paraformaldehyde-fixed cryostat, Vibratome and dewaxed tissue sections, at dilutions up to 1:4000 when used in combination with sensitive detection methods.



Figure 5: Cortical Lewy body in human brain immunostained using the rabbit antiserum to PGP9.5 (BML-PG9500). Micrograph courtesy of Prof. RJ Mayer (University of Nottingham).

[14] PGP 9.5 - a new marker for vertebrate neurons and neuroendocrine cells: R.J. Thompson, et al.; Brain Res. 278, 224 (1983)

[15] The immunolocalization of protein gene product 9.5 using rabbit polyclonal and mouse monoclonal antibodies: P.O. Wilson, et al.; Br. J. Exp. Pathol. 69, 91 (1988)

[16] Protein gene product (PGP) 9.5 in diagnostic (neuro-) oncology. An immunomorphological study: B. Ermisch & K. Schwechheimer; Clin. Neuropathol. 14, 130 (1995)

[17] The neuron-specific protein PGP 9.5 is a ubiquitin carboxyl-terminal hydrolase: K.D. Wilkinson, et al.; Science 246, 670 (1989)

[18] The structure of the human gene encoding protein gene product 9.5 (PGP9.5), a neuron-specific ubiquitin C-terminal hydrolase: I.N. Day, et al.; Biochem. J. 268, 521 (1990)

[19] Ubiquitin carboxyl-terminal hydrolase (PGP 9.5) is selectively present in ubiquitinated inclusion bodies characteristic of human neurodegenerative diseases: J. Lowe, *et al.*; J. Pathol. 161, 153 (1990)

[20] c-myc overexpression activates alternative pathways for intracellular proteolysis in lymphoma cells: R. Gavioli, et al.; Nat. Cell Biol. 3, 283 (2001)

# **Target/Substrate Proteins**

### Activating Enzymes (E1s) – Proteins

The regulation of all events in the NF- $\kappa$ B signalling pathway involves complex ubiquitin-mediated processes, both proteolytic and non-proteolytic. Similarly, involvement of both SUMO and NEDD8 pathways at different levels of the NF- $\kappa$ B pathway is also apparent together with the deconjugating and proteolytic machinery associated with both COP9 signalosome and proteasome-related complexes.

In the canonical pathway, NF- $\kappa$ B factors are retained in an inactive state by binding to the inhibitor of NF- $\kappa$ B (lkB) which, in response to cell stimulation, is ubiquitinylated (by derivatization with K<sup>48</sup>-linked chains) and degraded by the proteasome<sup>[21]</sup>. Prior to its ubiquitinylation, I $\kappa$ B is phosphorylated by the I $\kappa$ B kinase (IKK) complex. The IKK complex, consisting of two kinases IKK $\alpha$  and IKK $\beta$ , and the regulatory component NEMO, is activated by an upstream kinase (TAK1) which is in turn activated after TNF $\alpha$  or IL-1 receptor stimulation<sup>[22]</sup>.

[21] Ubiquitin signals in the NF-kappaB pathway: J. Terzic, *et al.*; Biochem. Soc. Trans. 35, 942 (2007) [22] Linear polyubiquitingtion: a new regulator of NF-kappaB activation: K. Jwai & F. Tokupaga: FMBO Rep. 10, 706 (

[22] Linear polyubiquitination: a new regulator of NF-kappaB activation: K. Iwai & F. Tokunaga; EMBO Rep. 10, 706 (2009)

### NF- $\kappa \textbf{B}$ and IKK $\alpha$ – Proteins

Product Name	Product #	Detail / Use	Size
$l\kappa B\alpha$ (human), (recombinant) (GST-tag)	BML-UW9970		50 µg
lκB $\alpha$ (human), (recombinant) (untagged)	BML-UW9975		50 µg
NF- $\kappa$ B (p50) (human), (recombinant) (His-tag)	ALX-201-285		2 µg
NF-ĸB p50 subunit (human) (35-381), (recombinant) (GST-tag)	BML-UW9980		50 µg
NF-ĸB p50 subunit (human) (35-381), (recombinant) (untagged)	BML-UW9985	Ubiquitinyiation/SUMUyiation substrates	50 µg
NF-κB (p65) (human), (recombinant) (His-tag)	ALX-201-284		2 µg
NF-κB p65 subunit (human) (12-317), (recombinant) (GST-tag)	BML-UW9990		50 µg
NF-κB p65 subunit (human) (12-317), (recombinant) (untagged)	BML-UW9995		50 µg

### NF- $\kappa\text{B}$ and IKK $\alpha$ – Antibodies

Product Name	Product #	Specificity	Application	Size
$l\kappa B\alpha$ monoclonal antibody (6A920)	ALX-804-209	Human, Mouse	FC, IHC, IP, WB	100 µg
[pSer32/36]I $\kappa$ B $\alpha$ polyclonal antibody	BML-SA412	Human, Mouse, Porcine	WB	20 µL / 100 µL
[pSer32/Ser36]I $\kappa$ B $\alpha$ monoclonal antibody (39A1413)	ADI-KAM-TF140	Human, Mouse, Rat, Bovine, Dog, Porcine	IP, WB	100 µg

### IKK $\alpha$ – Substrate

Product Name	Product #	Detail / Use	Size
$I\kappa B$ kinase substrate (biotinylated)	BML-P148	For use in IKK kinase assays with streptavidin-bound membranes	1 mg

#### p53 – Proteins

p53 is a much studied and complex multifunctional protein which plays a major role in the cellular response to DNA damage and other genomic aberrations. The activation of p53 can lead to either cell cycle arrest and DNA repair or apoptosis, through its involvement in cell cycle regulation as a trans-activator that acts to negatively regulate cell division by controlling a set of genes required for these processes. Activation and regulation of the p53 transcription pathway is controlled by a range of post-translational modifications. These include conjugation to ubiquitin and the ubiquitin-like proteins SUMO and NEDD8 via isopeptide bond formation at specific lysine residues, predominantly at the C-terminus.

In normal cells, p53 is maintained at a low level mainly through Hdm2-mediated ubiquitinylation and subsequent degradation by the proteasome. Hdm2 is a RING domain dependent ubiquitin E3 ligase that utilizes its C-terminal RING domain to promote not only p53 ubiquitinylation, predominantly at the C-terminus of p53, but also to target Hdm2 itself for auto-ubiquitinylation and subsequent degradation<sup>[23]</sup>. In contrast, SUMO and NEDD8 modifications have been shown to respectively activate and inhibit p53 transcriptional activity.

[23] p53 ubiquitination by Mdm2: a never ending tail?: A.S. Coutts, et al.; DNA Repair 8, 483 (2009)

Product Name	Product #	Detail / Use	Size
p53 (human), (recombinant) (His-tag)	BML-FW8820		20 µg
p53 (human), (recombinant) (GST-tag)	BML-FW9370	Ubiquitinylation/SUMUylation substrates	50 µg
p53 (368-386)	BML-P198	Substrate for assay of p300 and CBP HATs	2.5 mg
Hdm2 (catalytic RING domain) (human), (recombinant) (GST-tag)	BML-UW0200	Ubiquitinylation substrate	25 µg

### p53 – Antibodies and Detection Kits

Product Name	Product #	Specificity	Application	Size
IMMUNOSET® p53/MDM2 complex ELISA development set	ADI-960-070	Human, Mouse, Rat	ELISA	5 x 96 wells
p53 (human) monoclonal antibody (D0-7)	BML-PW1095	Human	ELISA, FC, IHC, IP, WB	25 µg
p53 (human) monoclonal antibody (EX-2)	BML-PW1100	Human	WB	25 µg
p53 (human) monoclonal antibody (EX-3)	BML-PW1115	Human	WB	25 µg
p53 (human) monoclonal antibody (EX-4)	BML-PW1110	Human	WB	25 µg
p53 (human) monoclonal antibody (PAb1801)	BML-PW1085	Human	ELISA, FC, IHC, IP, WB	25 µg
p53 (human) polyclonal antibody	ENZ-ABS195	Human	IHC (PS)	1 mL
p53 DINP1 SIP polyclonal antibody	ADI-905-300	Human, Mouse, Rat	IHC, WB	100 µg
p53 monoclonal antibody (BP53-12)	ALX-801-060	Human, Monkey	ELISA, ICC, IHC (PS), IP, WB	100 µg
p53 monoclonal antibody (BP53-12) (FITC conjugate)	ALX-801-060F	Human, Monkey	FC	100 µg
p53 monoclonal antibody (EX-1)	BML-PW1105	Human	WB	25 µg
p53 monoclonal antibody (PAb122)	ADI-KAM- CC002	Human, Mouse, Rat	FC, ICC, IHC, IP, WB	50 µg / 200 µg
p53 monoclonal antibody (PAb421)	BML-SA293	Human, Mouse, Rat	IHC, ICC, IP, WB	50 µg
p53 polyclonal antibody	ADI-905-510	Human	IHC	1 mL
p53 polyclonal antibody	ADI-KAP-CC030	Human, Mouse, Rat	IHC, IP, WB	250 µg
[pSer315]p53 monoclonal antibody (FPS315)	ADI-KAM- CC239	Human	WB	100 µg
[pSer392]p53 polyclonal antibody	BML-SA279	Human, Mouse, Rat	ICC, WB	5 µg / 25 µg

### p53 – Regulators

Product Name	Product #	Size
Nutlin-3	ALX-430-128	1 mg / 5 mg / 25 mg
p53 activator	BML-P603	500 µg

### **SUMOylation Substrates**

Covalent modification of proteins with SUMO affects many cellular processes including transcription, nuclear transport, DNA repair and cell cycle progression. Many hundreds of SUMO targets have been identified, although for the majority the function still remains unclear. It is possible to investigate the role of SUMOylation by mutating the relevant target lysine and observing a loss of function. However, such an approach may prove difficult since mapping of the modification site is problematic or mutation does not cause obvious change in phenotype. An alternative approach is to use a 'gain in modification' analysis by producing both SUMO-modified and -unmodified protein *in vitro* and comparing them in functional assays<sup>[24]</sup>. The following proteins may act as substrates for SUMO modification in combination with the necessary activating and conjugation enzymes.

[24] Preparation of sumoylated substrates for biochemical analysis. P. Knipscheer, et al.; Methods Mol. Biol. 497, 201 (2009)

Product Name	Product #	Size
IRF2 (human), (recombinant) (His-tag)	BML-UW0335	100 µg
PML SUMOylation motif (human), (recombinant) (GST-tag)	BML-UW9965	100 µg
RangaP1 fragment (human), (recombinant) (GST-tag)	BML-UW9755	100 µg
SP100 fragment (human), (recombinant) (GST-tag)	BML-UW9825	100 µg

### SUMOylation Substrates – Antibodies

Product Name	Product #	Specificity	Application	Size
RanGAP1 (human) polyclonal antibody	BML-PW8785	Human	WB	25 μL / 100 μL
Sp100 (human) polyclonal antibody	BML-PW0325	Human	ICC, WB	25 μL / 100 μL
Sp100 (SUMO modified) (human) polyclonal antibody	BML-PW0330	Human	ICC	25 μL / 100 μL
Huntingtin polyclonal antibody	BML-PW0595A	Mouse, Rat	WB	25 µg / 100 µg

### **NEDDylation Substrates – Antibodies**

Product Name	Product #	Specificity	Application	Size
CUL1 (Arabidopsis thaliana) polyclonal antibody	BML-PW0190	Arabidopsis thaliana	WB	25 μL / 100 μL

# **Detection & Isolation Kits & Components**

In pursuing the development of key reagents for the detection, isolation, purification, and characterization of components of the ubiquitin and ubiquitin-like protein cascades, Enzo Life Sciences has introduced a number of products of key utility. Prime examples include kits facilitating the study of ubiquitin and SUMO conjugation (Prod. No. BML-UW9920 and BML-UW8955), the isolation of mono- and polyubiquitinylated species (Prod. No. BML-UW8995), the investigation of ubiquitin binding parameters with various agarose-immobilized ubiquitin-binding domains, as well as the study in a variety of applications with a comprehensive range of Ubl-specific antibodies. The product range is now further extended by the addition of a number of agarose-immobilized ubiquitin-like proteins. Such matrices facilitate the specific isolation of those components within a system having an affinity for an ubiquitin-like protein or may be utilized in conjugation procedures to produce an agarose-immobilized complex.

### **Ubiquitin & Ubl Agarose Conjugates**

Product Name	Product #	Detail / Use	Size
FAT10 (human), (recombinant) (agarose immobilized)	BML-UW0140		0.5 mL
ISG15 (human), (recombinant) (agarose immobilized)	BML-UW0115		0.5 mL
NEDD8 (human), (recombinant) (agarose immobilized)	BML-UW0110		0.5 mL
SUMO-1 (human), (recombinant) (agarose immobilized)	BML-UW0095	Protein interaction studies	0.5 mL
SUM0-2 (human), (recombinant) (agarose immobilized)	BML-UW0100		0.5 mL
SUMO-3 (human), (recombinant) (agarose immobilized)	BML-UW0105		0.5 mL
Ubiquitin, (agarose immobilized)	BML-UW8630		0.5 mL

### **Ubiquitin-binding Domains**

Structurally distinct ubiquitin modifications, including mono-ubiquitinylation and up to eight types of polyubiquitin chains, enable ubiquitin to act as a multifunctional signal. This multifunctionality presupposes the existence of recognition factors that transduce the information contained in specific ubiquitin signals into appropriate downstream consequences.

The >16 thus far characterized ubiquitin-binding domains (UBDs) are rather small (20-150 amino acids) and diverge in both structure and patterns of ubiquitin recognition. A majority of the UBDs fold into alpha helical-based structures, including the UBA (ubiquitin-associated domain), UIM (ubiquitin-interacting motif), DUIM (doublesided ubiquitin-interacting motif), MIU (motif interacting with ubiquitin), CUE (coupling of ubiquitin conjugation to ER degradation), GAT (GGA: Golgi-localized, gamma-ear containing, ADP-ribosylation-factor-binding protein), and TOM (target of Myb) domains. Non-helical UBDs are also frequent and can be exemplified by the different ubiquitin-binding zinc fingers (ZnF) such as NZF (Npl4 zinc finger) and PAZ (polyubiquitin-associated zinc finger), the Ubc domain present in E2 enzymes, as well as the UEV (ubiquitin-conjugating enzyme variant), GLUE (GRAM-like ubiquitin-binding in Eap45), Jab1/MPN, and PFU (PLAA family ubiquitin-binding) domains. Besides their structural similarities, helical UBDs also share a common attraction to the same binding surface on the ubiquitin moiety, formed by the hydrophobic patch including and surrounding isoleucine 44 (Ile44). In contrast, ZnF-based UBDs, such as the A20-ZnF and the ZnF-UBP, display highly variable modes of ubiquitin recognition, which is in keeping with their highly divergent biological roles. Furthermore, while some UBDs appear to be strictly connected to a certain protein function, others fail to follow any general rules in correlation to functionality<sup>[25]</sup>.

[25] Functional roles of ubiquitin-like domain (ULD) and ubiquitin-binding domain (UBD) containing proteins. C. Grabbe et al.; Chem. Rev., 109, 1481-94 (2009)

## Ubiquitin-binding Domains – Proteins

Product Name	Product #	Detail / Use	Size
Dsk2 UBA domain, (agarose immobilized)	BML-UW9835	Binding studies of interacting proteins	0.5 mL
hHR23B UBA2 domain, (agarose immobilized)	BML-UW9440		0.5 mL
NBR1-derived UBA domain, (agarose immobilized)	BML-UW9445		0.5 mL
NUB1/NUB1L UBA domain, (agarose immobilized)	BML-UW9700		0.5 mL
p62-derived UBA domain, (agarose immobilized)	BML-UW9010		0.5 mL
Proteasome 19S Rpn10/S5a subunit (human), (recombinant) (GST-tag)	BML-UW8465		100 µg
Proteasome 19S Rpn10/S5a subunit (human), (recombi- nant) (GST-tag) (agarose immobilized)	BML-UW8635		0.5 mL
S5a UIM, (agarose immobilized)	BML-UW9820		0.5 mL
Ubiquitin binding entities, sampler pack	BML-UW0120		1 Pack
UQ1 UBA domain, (agarose immobilized)	BML-UW9830		0.5 mL
VPS9-derived CUE domain, (agarose immobilized)	BML-UW9450		0.5 mL

# Ubiquitin-binding Domains – Antibodies

Product Name	Product #	Specificity	Application	Size
p62 (human) polyclonal antibody	BML-PW9860	Human	ICC, WB	25 μL / 100 μL
NUB1 (human) polyclonal antibody	BML-PW9685	Human	WB	25 μL / 100 μL

### **Detection, Isolation and Modification Kits**

While the ubiquitin and Ubl signaling pathways are somewhat complex, there is much information that can be gleaned from careful study of individual components. In addition to its range of fundamental reagents, Enzo Life Sciences offers a number of kits designed to facilitate more detailed investigation in a consistent and reproducible fashion. A ubiquitinylation kit (Prod. No. BML-UW9920) provides the means for generating a range of thioester-linked ubiquitin conjugation enzymes (E2s), utilizing the first two steps in the ubiquitin cascade, for use in the transfer of ubiquitin to E3 ligases and the subsequent ubiquitinylation of target/substrate proteins. Similarly, a SUMOylation kit (Prod. No. BML-UW8955) provides a means of generating SUMOylated proteins *in vitro* using the SUMO enzyme cascade. A NEDDylation kit (Prod. No. BML-UW0590) is also available for study of the NEDD8 cascade.

Product Name	Product #	Utility	Size				
UBIQUITIN							
Auto-ubiquitinylation kit	BML-UW0970	Testing of proteins for auto-ubiquitinylation activity	10 Tests				
UBI-QAPTURE-Q® kit	BML-UW8995	Isolation and enrichment of ubiquitinylated proteins	20 Tests				
Ubiquitin activating kit	BML-UW0400A	Activation of ubiquitin for use in ubiquitinylation experiments	20 Tests				
Ubiquitin conjugating kit (HeLa lysate-based)	BML-UW9915	Generating ubiquitinylated proteins	20 Tests				
Ubiquitinylation kit	BML-UW9920	Generating ubiquitin-E2 thioesters	20 Tests				
SUMO							
SUMO-QAPTURE-T® kit	BML-UW1000A	Isolation and enrichment of SUMOylated proteins	10 Tests				
SUMOylation kit	BML-UW8955	Generating SUMOylated proteins	20 Tests				
NEDD8							
NEDDylation kit	BML-UW0590	Generating NEDD8-E2 thioesters	1 Kit				

#### **SUMOylation Kit**

This kit provides a means of generating SUMOylated proteins *in vitro* using the SUMOylation enzyme cascade. A short sequence containing the consensus  $\Psi$ -K-X-D/E (where lysine is the amino acid modified,  $\Psi$  is a large hydrophobic residue and X is any amino acid residue) is thought to be necessary for this *in vitro* protein SUMOylation; however, SUMOylation has also been observed in cases where the consensus site is absent. A control target protein is provided together with all other necessary components. SUMO-specific antibodies are provided for detection of SUMOylated proteins. The kit contains sufficient material for 20 x 20 µL reactions.

#### Suggested Uses:

- · For SUMO-modification of specific proteins in vitro
- To demonstrate that novel proteins are potential targets for SUMOylation under *in vitro* conditions
- To generate substrates for deSUMOylating enzymes, such as SENP1 and SENP2
- To test proteins for SUMO E3 ligase activity



Figure 6: Western blots following SDS-PAGE of SUMOylation assays using: [A] RANGAP1 (BML-UW9755); [B] SP100 (BML-UW9825); and [C] p53 (BML-FW9370) as substrate proteins with the three SUMOs assayed in the presence (+) and absence (-) of ATP- lane 1: SUMO-1 (BML-UW9195); lane 2: SUMO-2 (BML-UW9205); and lane 3: SUMO-3 (BML-UW9215). Detection was with the appropriate SUMO antibodies (SUMO-1: BML-PW9460, SUMO-2/3: BML-PW9465).

### **UBI-QAPTURE-Q® Kit**

The UBI-QAPTURE-Q<sup>®</sup> Kit was specifically developed for the isolation and enrichment of ubiquitinylated proteins. The kit facilitates the isolation of both mono- and poly-ubiquitinylated proteins (independent of lysine residue chain linkage) from cell extracts, tissue lysates and *in vitro* assay solutions through the use of a broad spectrum affinity matrix. Captured proteins may be analyzed by Western blotting using the highly sensitive ubiquitin-conjugate specific antibody provided, using antibodies to specific proteins of interest, or eluted from the matrix for subsequent biochemical characterization. The UBI-QAPTURE-Q<sup>®</sup> matrix supplied with the kit has superior binding characteristics compared to other commercially available matrices and is compatible with a wide range of lysate buffers and cell/tissue samples from a variety of species. The kit provides sufficient material for approximately 20 binding assays.

#### Suggested Uses:

- Isolation and detection of ubiquitinylated protein conjugates from a specific cell/tissue lysate
- Capture and analysis of specific ubiquitinylated protein conjugates of interest from particular cell/tissue lysates
- Separation of ubiquitinylated/non-ubiquitinylated forms of specific proteins of interest
- Release of free proteins in their active/native form by cleavage of ubiquitin/ubiquitin chains from the UBI-QAPTURE-Q<sup>®</sup> matrix using a deubiquitinylating enzyme
- Release of ubiquitinylated proteins in their active/native form by elution from the UBI-QAPTURE-Q<sup>®</sup> matrix using high salt buffer



Figure 7: Schematic overview of UbiQapture Kit isolation and detection process.

Figure 8: Western blot analysis demonstrating ubiquitin enrichment of partially purified and lysatederived ubiquitinylated proteins after UBI-QAPTURE-Q<sup>®</sup>. Ubiquitin-protein conjugates present in starting material, unbound fraction and elution fraction were detected by western blotting using the provided

ubiquitin-conjugate specific HRP-linked antibody at a dilution of 1:1000 dilution. A: Capture of ubiquitinylated UbcH5a from *in vitro* ubiquitinylation assay. B: Capture of Ub-protein conjugates from control ubiquitinylated-protein lysate (BML-UW0130).

- Key: SM = Starting Material, UF = Unbound Fraction
  - EL = Elution Fraction



Product Name	Product #	Utility	Size
10X SUMOylation kit buffer	BML-KW9890	Assay buffer from the SUMOylation Kit	5 mL
10X Ubiquitinylation kit buffer	BML-KW9885	Assay buffer from the Ubiquitinylation kit	5 mL
ATP (energy) Regeneration Solution	BML-EW9810	To facilitate efficient conjugation and degradation studies	100 µL
Fraction I (HeLa)	BML-HW8600	For ubiquitinylation assays and <i>in vitro</i> conjugation	1 mg
Fraction II (HeLa)	BML-HW8605	experiments	1 mg
HeLa S100 fraction	BML-SW8750	For demonstrating ubiquitin-proteasome mediated conjuga- tion/degradation	1 mg
Mg <sup>2+</sup> /ATP Activating Solution	BML-EW9805	To facilitate efficient conjugation and degradation studies	100 µL

# **Proteasome & Related Complexes**

Enzo Life Sciences has an extensive listing of reagents for investigation of the proteasome and related multi-subunit complexes possessing various catalytic activities. These complexes include the proteasome in its various forms (30S, 26S, 20S, 19S, 11S, and chimeras thereof), the COP9 signalosome, TPPII and other post-proteasomal processing enzymes.

### **11S Activator – Proteins**

Product Name	Product #	Size
Proteasome 11S $\alpha$ subunit (human), (recombinant) (GST-tag)	BML-PW9120	100 µg
Proteasome 11S $\beta$ subunit (human), (recombinant) (GST-tag)	BML-PW9125	100 µg
Proteasome 11S $\gamma$ subunit (human), (recombinant) (GST-tag)	BML-PW9130	100 µg
Proteasome activator 11S complex (human), (purified)	BML-PW9420	25 µg
Proteasome activator 11S $\alpha$ subunit (human), (recombinant)	BML-PW9865	100 µg
Proteasome activator 11S $\beta$ subunit (human), (recombinant)	BML-PW9870	100 µg
Proteasome activator 11S $\gamma$ subunit (human), (recombinant)	BML-PW9875	100 µg

### 11S Activator – Antibodies

Product Name	Product #	Specificity	Application	Size
Proteasome activator 11S $\alpha$ subunit polyclonal antibody	BML-PW8185	Human, Mouse, Rat	IHC, WB	25 μL / 100 μL
Proteasome activator 11S $\beta$ subunit polyclonal antibody	BML-PW8240	Human, Mouse, Rat	IHC, WB	25 μL / 100 μL
Proteasome activator 11S $\gamma$ subunit polyclonal antibody	BML-PW8190	Human, Mouse	IHC, WB	25 μL / 100 μL
Proteasome activator 11S subunit antibody sampler pack	BML-PW8915	Human, Mouse	IHC, WB	3 x 10 µL

### **Miscellaneous Activator Complexes – Antibodies**

Product Name	Product #	Specificity	Application	Size
Blm10 (Saccharomyces cerevisiae) polyclonal antibody	BML-PW0570	Saccharomyces cervisiae	WB	25 µL
POMP polyclonal antibody	BML-PW9715	Human, Rat, Monkey	IHC, IP, WB	25 μL / 100 μL

# 19S Regulator ATPase Subunits – Antibodies

Product Name	Product #	Specificity	Application	Size
Proteasome 19S (ATPase subunit) antibody sampler pack (human)	BML-PW8935	Human	IHC, IP, WB	10 x 10 µL
Proteasome 19S (ATPase subunit) antibody sampler pack (yeast)	BML-PW8940	Yeast	IHC, IP, WB	8 x 10 µL
Proteasome 19S Rpt1/S7 subunit (human) monoclonal antibody (MSS1-104)	BML-PW8825	Human, Mouse	WB	25 μL / 100 μL
Proteasome 19S Rpt1/S7 subunit (human) monoclonal antibody (MSS1-92)	BML-PW9400	Human	IP	25 µL
Proteasome 19S Rpt1/S7 subunit (human) polyclonal antibody	BML-PW8315	Human	WB	25 µL / 100 µL
Proteasome 19S Rpt1/S7 subunit (yeast) polyclonal antibody	BML-PW8255	Yeast	WB	25 μL / 100 μL
Proteasome 19S Rpt1/S7 subunit polyclonal antibody	BML-PW8165	Human, Yeast	WB	25 µL / 100 µL
Proteasome 19S Rpt2/S4 (mts2) subunit polyclonal antibody	BML-PW8160	Wide range of species	IHC, WB	25 µL / 100 µL
Proteasome 19S Rpt2/S4 subunit (yeast) polyclonal antibody	BML-PW8260	Yeast	WB	25 μL / 100 μL
Proteasome 19S Rpt2/S4 subunit polyclonal antibody	BML-PW8305	Human, Mouse	WB	25 μL / 100 μL
Proteasome 19S Rpt2/S4 subunit polyclonal antibody	BML-PW0445	Arabidopsis	WB	25 µL / 100 µL
Proteasome 19S Rpt3/S6b subunit monoclonal antibody (TBP7-27)	BML-PW8765	Human, Rabbit	IHC, IP, WB	25 μL / 100 μL
Proteasome 19S Rpt3/S6b subunit polyclonal antibody	BML-PW8250	Human, Yeast	IHC, WB	25 μL / 100 μL
Proteasome 19S Rpt3/S6b subunit polyclonal antibody	BML-PW8175	Human, Mouse, Rat, Cow	IHC, WB	25 μL / 100 μL
Proteasome 19S Rpt4/S10b subunit monoclonal antibody (p42-23)	BML-PW8830	Human, Mouse	WB	25 µL / 100 µL
Proteasome 19S Rpt4/S10b subunit polyclonal antibody	BML-PW8220	Human, Yeast	IHC, WB	25 μL / 100 μL
Proteasome 19S Rpt5/S6a subunit monoclonal antibody (TBP1-19)	BML-PW8770	Human, Mouse, Rat, Rabbit	IHC, WB	25 μL / 100 μL
Proteasome 19S Rpt5/S6a subunit polyclonal antibody	BML-PW8245	Yeast	WB	25 μL / 100 μL
Proteasome 19S Rpt5/S6a subunit polyclonal antibody	BML-PW8375	Arabidopsis, Cauliflower	IHC, WB	25 μL / 100 μL
Proteasome 19S Rpt5/S6a subunit polyclonal antibody	BML-PW8310	Human	WB	25 μL / 100 μL
Proteasome 19S Rpt6/S8 subunit (human) polyclonal antibody	BML-PW8320	Human	WB	25 μL / 100 μL
Proteasome 19S Rpt6/S8 subunit monoclonal antibody (p45-110)	BML-PW9265	Human, Mouse, Rat	IHC, IP, WB	25 μL / 100 μL
Proteasome 19S Rpt6/S8 subunit polyclonal antibody	BML-PW8215	Human, Yeast	IHC, WB	25 μL / 100 μL

### 19S Regulator non-ATPase Subunits – Protein

Product Name	Product #	Specificity	Size
Gankyrin, His <sub>6</sub> -tagged	BML-UW9815	A proteasome-interacting protein	100 µg
Rpn10 (S5a) (human), (recombinant) (His-tag)	BML-UW1065	A ubiquitin-interacting protein	50 µg

### 19S Regulator non-ATPase Subunits – Antibodies

Product Name	Product #	Specificity	Application	Size
Gankyrin (human) polyclonal antibody	BML-PW8325	Human	WB	25 μL
Proteasome 19S (non-ATPase subunit) antibody sampler pack (human)	BML-PW8965	Human	WB	7 x 100 µL
Proteasome 19S Rpn10/S5a subunit monoclonal antibody (S5a-18)	BML-PW9250	Human	IHC, IP, WB	25 μL / 100 μL
Proteasome 19S Rpn11/S13 subunit (human) polyclonal antibody	BML-PW9625	Human	IHC, WB	25 μL / 100 μL
Proteasome 19S Rpn12/S14 subunit (human) monoclonal antibody (P31-27)	BML-PW8835	PW8835 Human I		25 μL / 100 μL
Proteasome 19S Rpn12/S14 subunit (human) monoclonal antibody (P31-38)	BML-PW9260	/IL-PW9260 Human		25 μL / 100 μL
Proteasome 19S Rpn12/S14 subunit (human) polyclonal antibody	BML-PW8815	Human	IHC, WB	25 μL / 100 μL
Proteasome 19S Rpn12/S14 subunit polyclonal antibody	BML-PW0440	Arabidopsis, Cauliflower	WB	25 μL
Proteasome 19S Rpn2/S1 subunit (human) monoclonal antibody (112-1)	BML-PW9270	Human	WB	25 μL / 100 μL
Proteasome 19S Rpn5 subunit polyclonal antibody	BML-PW0450	S. Cerevisiae, A. Thaliana	WB	25 μL
Proteasome 19S Rpn6/S9 subunit polyclonal antibody	BML-PW8370	Arabidopsis, Cauliflower	WB	25 μL / 100 μL
Proteasome 19S Rpn7/S10a subunit polyclonal antibody	BML-PW8225	Human, Yeast	IP, WB	25 μL / 100 μL
Proteasome 19S Rpn8/S12 subunit (human) polyclonal antibody	BML-PW8180	Human	IHC, WB	25 μL

## Proteasome 20S Complex – Proteins

Product Name	Product #	Utility	Size
Immunoproteasome 20S (human), (purified)	BML-PW9645	Isolated from human spleen	50 µg
Proteasome 20S (human), (purified)	BML-PW8720	Isolated and purified from human erythrocytes	50 µg
Proteasome 20S (Saccharomyces cerevisiae)	BML-PW8775	Purified from Saccharomyces cerevisiae	50 µg

#### **Proteasome-associated Proteins – Antibodies**

Product Name	Product #	Specificity	Application	Size
BAG-1 (mouse) (CT) polyclonal antibody (Bur 1702)	ALX-210-009	Mouse	ihc, ip, wb	50 µL
PAC1 (human) monoclonal antibody (EX-5)	BML-PW0480	Human	IP, WB	25 µg / 100 µg
PAC2 (human) monoclonal antibody (EX-6)	BML-PW0485	Human	IP, WB	25 µg / 100 µg
PAC3 (human) monoclonal antibody (EX-7)	BML-PW0490	Human	IP, WB	25 µg / 100 µg
PBA1 (Arabidopsis thaliana) polyclonal antibody	BML-PW0430	Arabidopsis	WB	25 μL / 100 μL
PBF1 (Arabidopsis thaliana) polyclonal antibody	BML-PW0435	Arabidopsis	WB	25 μL / 100 μL
PI31 polyclonal antibody	BML-PW9710	Human, Mouse	WB	25 μL / 100 μL

### **Proteasome 20S Assay Kits**

& L. T. Sorell; J. Immunol. Methods. 334, 91-103 (2008)

K. Egerer, et al.; J. Rheumatol. 29, 2045-2052 (2002)

C. Jakob, et al.; Blood. 109, 2100-2105 (2007)

### Proteasome ELISA Kit (BML-PW0575)

Proteasomes are non-lysosomal proteolytic complexes localized primarily in the cytoplasm and in the nucleus of eukaryotic cells<sup>[26]</sup>. In patients suffering from autoimmune diseases, malignant myelo-proliferative syndromes, multiple myeloma, acute and chronic lymphocytic leukaemia, solid tumors, sepsis or trauma, the concentration of circulating proteasomes has been found to be elevated, correlating with the disease state and having possible prognostic significance <sup>[27-29]</sup>.

This kit provides the means to quantify proteasome concentration in biological samples using a Sandwich ELISA technique, utilizing two proteasome subunit specific antibodies for capture and detection purposes, together with a highly sensitive substrate. Sample proteasome levels are determined by comparison to a 20S proteasome calibration curve produced in parallel. This kit provides sufficient material for a single 96-well plate.

[26] Immunological methods to quantify and characterize proteasome complexes: development and application: Majetschak

[27] Serum concentration and localization in tumor cells of proteasomes in patients with hematologic malignancy and their

[28] Circulating proteasomes are markers of cell damage and immunologic activity in autoimmunediseases:

[29] Circulating proteasome levels are an independent prognostic factor for survival in multiple myeloma:

pathophysiologic significance: M. Wada, et al.; J. Lab. Clin. Med. 121, 215-223 (1993)



#### Applications:

- Determination of proteasome levels in biological samples (cell lysates, tissue extracts, plasma, serum)
- Comparison of proteasome levels in plasma/serum samples associated with a particular disease/illness with samples from healthy controls
- Investigation of variation in proteasome levels in abnormal cell lines/tissues

Product Name	Product #	Utility	Size
Proteasome 20S assay kit	BML-AK740	Fluorogenic, non-radioactive assay for screening inhibitors and modulators of the 20S proteasome	96 wells

### Proteasome 20S $\alpha\text{-Subunits}$ – Antibodies

Product Name	Product #	Specificity	Application	Size
Proteasome 20S core subunits polyclonal antibody	BML-PW8155	Human, Mouse, Rat, Rabbit, Yeast	IHC, IP, WB	25 μL / 100 μL
Proteasome 20S core subunits polyclonal antibody	BML-PW9355	Yeast	WB	25 μL/100 μL
Proteasome 20S ( $\alpha$ subunits) antibody sampler pack	BML-PW8900	Varies	IHC, IP, WB	8 x 10 µL
Proteasome 20S ( $\alpha$ subunits) antibody sampler pack (for immunofluorescence)	BML-PW8925	Varies	IHC, IP, WB	4 x 25 µg
Proteasome 20S $\alpha$ subunit (human) monoclonal antibody (HP103)	BML-PW8275	Human	IHC	100 µg
Proteasome 20S $\alpha$ subunit (human) monoclonal antibody (HP305)	BML-PW8280	Human	IHC	100 µg
Proteasome 20S $\alpha$ subunit (human) monoclonal antibody (HP810)	BML-PW8265	Human	IHC, IP	100 µg
Proteasome 20S $\alpha$ subunit (human) monoclonal antibody (HP903)	BML-PW8270	Human	IHC, IP	100 µg
Proteasome 20S $\alpha$ 1, 2, 3, 5, 6 & 7 subunits monoclonal antibody (MCP231)	BML-PW8195	Human, Rabbit, Rat, Mouse, Yeast, Potato	IHC, WB	25 µL / 100 µL
Proteasome 20S $\alpha 2$ subunit monoclonal antibody (MCP21)	BML-PW8105	Human, Rabbit, Cow	IHC, IP, WB	25 μL / 100 μL
Proteasome 20S $\alpha 2$ subunit monoclonal antibody (MCP21) (agarose immobilized)	BML-PW8335	Human, Rabbit, Cow	WB	0.5 ml
Proteasome 20S $\alpha$ 2 subunit monoclonal antibody (MCP236)	BML-PW9385	Human, Mouse	WB	25 µL
Proteasome 20S $\alpha 3$ subunit monoclonal antibody (MCP257)	BML-PW8115	Human, Mouse, Rat, Rabbit	IHC, WB	25 μL / 100 μL
Proteasome 20S $\alpha 4$ subunit (human) monoclonal antibody (MCP34)	BML-PW8120	Human	IHC, IP, WB	25 μL / 100 μL
Proteasome 20S $\alpha 4$ subunit (human) monoclonal antibody (MCP34) (agarose immobilized)	BML-PW9005	Human	WB	0.5 mL
Proteasome 20S $\alpha$ 4 subunit monoclonal antibody (MCP79)	BML-PW9140	Human, Mouse	IHC, IP, WB	25 μL / 100 μL
Proteasome 20S $\alpha$ 5 subunit monoclonal antibody (MCP196)	BML-PW8125	Human, Mouse, Rat, Rabbit	IHC, WB	25 µL / 100 µL
Proteasome 20S $\alpha$ 6 subunit monoclonal antibody (MCP106)	BML-PW9390	Human, Mouse, Rabbit	WB	1 mL
Proteasome 20S $\alpha$ 6 subunit monoclonal antibody (MCP20)	BML-PW8100	Human, Rabbit	IHC, IP, WB	25 µL / 100 µL
Proteasome 20S $\alpha$ 7 subunit monoclonal antibody (MCP72)	BML-PW8110	Human, Rat, Rabbit, Yeast, Arthropod	IHC, WB	25 µL / 100 µL

# Proteasome 20S $\beta\mbox{-Subunits}$ – Antibodies

Product Name	Product #	Specificity	Application	Size
Proteasome 20S ( $\beta$ subunits) antibody sampler pack	BML-PW8905	Varies	IHC, IP, WB	12 x 10 µL
Proteasome 20S core subunits polyclonal antibody	BML-PW8155	Human, Mouse, Rat	ihc, ip, wb	25 µL / 100 µL
Proteasome 20S core subunits polyclonal antibody	BML-PW9355	Yeast	WB	25 µL / 100 µL
Proteasome 20S $\beta$ 1 subunit monoclonal antibody (MCP421)	BML-PW8140	Human, Rabbit, Yeast	IHC, WB	25 µL / 100 µL
Proteasome 20S $\beta$ 1i subunit (human) polyclonal antibody	BML-PW8345	Human	IHC, WB	25 µL / 100 µL
Proteasome 20S $\beta$ 1i subunit monoclonal antibody (LMP2-13)	BML-PW8840	Human, Rat	IHC, WB	25 µL / 100 µL
Proteasome 20S $\beta$ 1i subunit polyclonal antibody	BML-PW8205	Human, Mouse, Rat	IHC, WB	25 μL / 100 μL
Proteasome 20S $\beta$ 2 subunit monoclonal antibody (MCP165)	BML-PW9300	Human, Mouse	IHC, WB	25 μL / 100 μL
Proteasome 20S $\beta$ 2 subunit monoclonal antibody (MCP168)	BML-PW8145	Human, Yeast	IHC, WB	25 μL / 100 μL
Proteasome 20S $\beta 2/\beta 2i$ subunit polyclonal antibody	BML-PW8210	Human, Mouse, Yeast	IHC, WB	25 µL / 100 µL
Proteasome 20S $\beta$ 2i subunit (human) polyclonal antibody	BML-PW8350	Human	WB	25 µL / 100 µL
Proteasome 20S $\beta$ 2i subunit polyclonal antibody	BML-PW8150	Human, Mouse	IHC, WB	25 µL / 100 µL
Proteasome 20S $\beta$ 3 subunit monoclonal antibody (MCP102)	BML-PW8130	Human, Rabbit, Rat, Mouse	IHC, WB	25 µL / 100 µL
Proteasome 20S $\beta$ 4 subunit polyclonal antibody	BML-PW8890	Human, Mouse, Rat	WB	25 µL / 100 µL
Proteasome 20S $\beta$ 5 subunit polyclonal antibody	BML-PW8895	Human	IHC, WB	25 µL / 100 µL
Proteasome 20S $\beta$ 5i subunit (human) polyclonal antibody	BML-PW8355	Human	IHC, WB	25 μL / 100 μL
Proteasome 20S $\beta$ 5i subunit monoclonal antibody (LMP7-1)	BML-PW8845	Human, Rat	IHC, WB	25 μL / 100 μL
Proteasome 20S $\beta$ 6 subunit polyclonal antibody	BML-PW9000	Human, Rat, Mouse	IHC, WB	25 μL / 100 μL
Proteasome 20S $\beta$ 7 subunit monoclonal antibody (MCP205)	BML-PW8135	Human, Rabbit	IHC, WB	25 μL / 100 μL
Proteasome 20S $\beta$ 7 subunit monoclonal antibody (MCP219)	BML-PW9395	Human, Mouse	WB	25 μL / 100 μL
Proteasome 20S $\beta$ 7 subunit monoclonal antibody (MCP444)	BML-PW9150	Human	IP, WB	25 µL / 100 µL

## Proteasome Inhibitors

Product Name	Chymotrypsin-like	Trypsin-like	Caspase-like	Product #	Size
Ac-Ala-Pro-Nle-Asp-H			•	BML-AW9485	100 µg
N-Acetyl-Leu-Leu-Methional	•		•	BML-PI100	5 mg / 25 mg
Ac-Leu-Leu-Nie-CHO	•		•	BML-P120	5 mg / 25 mg
Aclacinomycin A (Aclarubicin)	•			BML-AW8655	5 mg
Ada-(Ahx) <sub>3</sub> -(Leu) <sub>3</sub> -vinyl sulfone	•		•	BML-AW9155	100 µg
Ada-Tyr-(Ahx) <sub>3</sub> -(Leu) <sub>3</sub> -vinyl sulfone	•	•	•	BML-AW9160	100 µg
Bactenecin-5 precursor peptide	•	•	•	BML-BW9315	100 µg
Celastrol	•	•		ALX-350-332	5 mg / 25 mg
(-)-Epigallocatechin gallate (EGCG)	•			ALX-270-263	10 mg / 50 mg
Epoxomicin	•			BML-PI127	100 µg
Gliotoxin	•			BML-PI129	2 mg / 10 mg
Lactacystin (native)	•	•		ALX-350-245	0.1 mg / 0.5 mg / 1 mg
Lactacystin	•	•		BML-PI104	200 µg / 1 mg
<i>clasto</i> -Lactacystin $\beta$ -lactone	•	•		BML-PI108	100 µg
N-Tosyl-Lys-chloromethylketone (TLCK)		•		BML-PI121	200 mg
PR11	•	•		BML-PW9325	100 µg
PR26	•	•		BML-PW9790	100 µg
PR39 propeptide	•	•		BML-PW8850	100 µg
Proteasome inhibitor II (aldehyde)	•			ALX-260-090	1 mg / 5 mg
Proteasome inhibitor pack	•	•		BML-PW9901	1 Pack
Z-Leu-Leu-B(OH) <sub>2</sub> (MG262)	•			BML-PI109	100 µg
Z-Leu-Leu-H (MG132)	•			BML-PI102	5 mg / 25 mg
Z-Leu-Leu-vinyl sulfone	•	•		BML-ZW9170	500 µg
Z-Leu-Leu-Tyr-ketoaldehyde	•			BML-ZW8655	5 mg
Z-Pro-Nie-Asp-CHO	•			BML-ZW9490	100 µg

### Proteasome Substrates

Product Name	Chymotrypsin-like	Trypsin-like	Caspase-like	Product #	Size
Ac-Arg-Leu-Arg-AMC		•		BML-AW9785	5 mg
Ac-Gly-Pro-Leu-Asp-AMC			•	BML-AW9560	5 mg
Ac-NIe-Pro-NIe-Asp-AMC			•	BML-AW9555	5 mg
Boc-Leu-Arg-Arg-AMC		•		BML-BW8515	5 mg
Bz-Val-Gly-Arg-AMC		•		BML-BW9375	5 mg
MCMV pp89 substrate peptide	•	•		BML-PW9380	100 µg
Suc-Arg-Pro-Phe-His-Leu-Leu-Val-Tyr-AMC	•			BML-SW8525	5 mg
Suc-Leu-Leu-Val-Tyr-AMC	•			BML-P802	5 mg
Z-Leu-Leu-Glu-AMC			•	BML-ZW9345	5 mg
Z-Leu-Leu-Glu-βNA			•	BML-ZW8520	5 mg
Z-Gly-Gly-Leu-AMC	•			BML-ZW8505	5 mg
Z-Gly-Gly-Leu-βNA	•			BML-ZW8510	5 mg
Proteasome substrate I (fluorogenic)	•			ALX-260-088	1 mg / 5 mg
Proteasome substrate IV (fluorogenic)	•			ALX-260-087	1 mg / 5 mg
Proteasome substrate pack	•	•	•	BML-PW9905	1 Pack

### Proteasome 26S Proteins & Kits

Product Name	Product #	Utility	Size
Proteasome 26S (human), (purified)	BML-PW9310	Highly purified preparation of '26S' proteasomes useful for carrying out <i>in vitro</i> protein degradation studies with suitably ubiquitinylated protein substrates.	50 µg
Proteasome 26S degradation kit	BML-PW8950	This kit contains a highly purified, human erythrocyte derived, preparation of '26S' proteasomes useful for carrying out <i>in vitro</i> protein degradation studies with suitably ubiquitinylated protein substrates. The preparation consists of a high purity mixture of '26S' proteasomes singly (26S) and doubly (30S) capped with 19S regula- tory subunit complexes in the ratio of 40% single cap : 60% double capped at the time of preparation. Additional kit components include ATP for proteasomal activation. Quantity: 96 wells.	1 Kit
Proteasome ELISA Kit	BML-PW0575	This kit provides the means to quantify proteasome concentrations in biological samples using a Sandwich ELISA technique, utilizing two proteasome subunit-specific antibodies for capture and detection purposes, together with a highly sensitive substrate. Quantity: 96 wells.	1 Kit

## COP9 Signalosome CSN – Protein

Product Name	Product #	Utility	Size
COP9 signalosome (human), (purified)	BML-PW9425	Protein complex with isopeptidase activity	20 µg

## COP9 Signalosome CSN – Antibodies

Product Name	Product #	Specificity	Application	Size
COP9 signalosome Csn1 subunit polyclonal antibody	BML-PW8285	Human, Mouse, Pig	IHC, IP, WB	25 µL / 100 µL
COP9 signalosome Csn2 subunit polyclonal antibody	BML-PW8230	Human	IHC, IP, WB	25 μL / 100 μL
COP9 signalosome Csn2 subunit (mouse) polyclonal antibody	BML-PW9720	Mouse	IP, WB	25 µL
COP9 signalosome Csn3 subunit polyclonal antibody	BML-PW8235	Human	IHC, WB	25 μL / 100 μL
COP9 signalosome Csn4 subunit polyclonal antibody	BML-PW8360	Human, <i>Arabidopsis</i> , Cauliflower	WB	25 μL / 100 μL
COP9 signalosome Csn5 subunit polyclonal antibody	BML-PW8365	Arabidopsis, Cauliflower	WB	25 μL / 100 μL
COP9 signalosome Csn6 subunit (human) polyclonal antibody	BML-PW8295	Human	WB	25 μL / 100 μL
COP9 signalosome Csn7 subunit (human) polyclonal antibody	BML-PW8300	Human	IP, WB	25 μL / 100 μL
COP9 signalosome Csn8 subunit polyclonal antibody	BML-PW8290	Human, Mouse, Pig, <i>Xenopus</i>	IHC, IP, WB	25 μL / 100 μL
COP9 Signalosome subunits antibody sampler pack	BML-PW8945	All of above	IHC, IP, WB	8 x 10 µL

### **TPPII – Protein**

Product Name	Product #	Utility	Size
TPPII complex (human), (purified)	BML-PW9660	Enzyme with endopeptidase and exopeptidase activity	10 µg

### **TPPII – Antibody**

Product Name	Product #	Specificity	Application	Size
Tripeptidyl Peptidase II (human) polyclonal antibody	BML-PW0690	Human	WB	25 μL / 100 μL

### p97 – Antibody

Product Name	Product #	Specificity	Application	Size
Valosin-containing protein polyclonal antibody	BML-PW9335	Human, Mouse, Rat, Pig	WB	25 µL

## Autophagy – Proteins

Product Name	Product #	Utility	Size
GABARAP (human), (recombinant) (GST-tag)	BML-UW1175		500 µg
GABARAP-L2 (human), (recombinant) (GST-tag)	BML-UW1195	Use in general and selective autophagy studies	500 µg
LC3B (human), (recombinant) (GST-tag)	BML-UW1155		500 µg
LC3-I (human), (recombinant) (His-tag)	ADI-APR-100	Western blot control	50 µg / 200 µg
p62 (human), (recombinant) (GST-tag)	ENZ-PRT120	Regulator of the degradation of ubiquitinylated proteins	50 µg
NBR1-derived UBA domain (agarose immobilized)	BML-UW9445	Use in general and selective autophagy studies	0.5 mL

# Autophagy – Antibodies and Detection Kits

Product Name	Product #	Specificity	Application	Size
CYTO-ID® Autophagy detection kit	ENZ-51031	Species Independent	FC, MC, MP	50 Tests / 200 Tests
LC3 monoclonal antibody (2G6)	ALX-803-081	Human	ICC, WB	100 µg
LC3 monoclonal antibody (2G6) (fluorescein labeled)	BML-PW1205	Human	ICC	25 µL
LC3 monoclonal antibody (5H3)	ALX-803-082	Human	ICC, IHC, WB	100 µg
LC3B monoclonal antibody (5F10)	ALX-803-080	Human, Mouse, Rat, Dog, Hamster	ICC, WB	100 µg
NBR1 (human) polyclonal antibody	BML-PW1125	Human	WB	25 μL / 100 μL
NBR1 (human) polyclonal antibody (fluorescein labeled)	BML-PW1130	Human	ICC	25 µL
p62 ELISA kit	ADI-900-212	Human, Mouse, Rat	ELISA	96 wells
PROTEOSTAT® Aggresome detection kit	ENZ-51035	Species Independent	FC, MC	25 Tests / 100 Tests



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