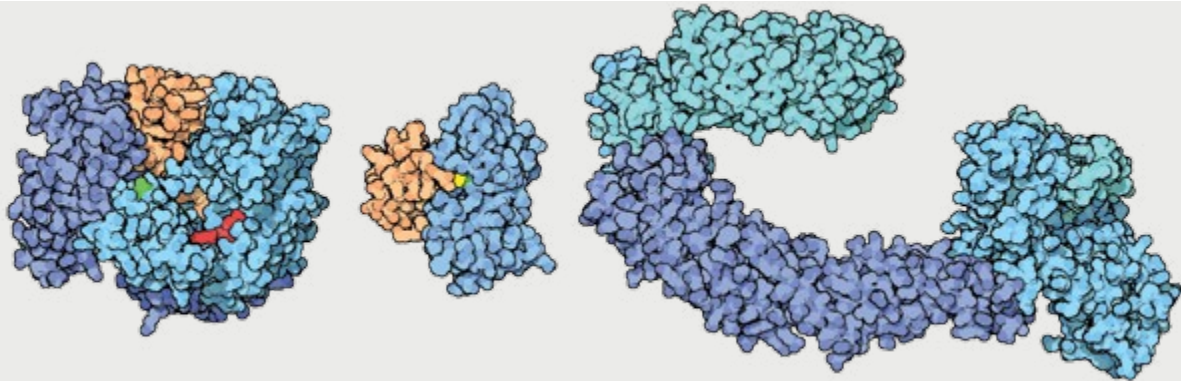




UBIQUITIN & UBL SIGNALING



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
K⁶³-linkage-specific Ubiquitin-reactive Antibody
Blocking Peptides for Ubl-reactive Antibodies

Ubiquitin Remnant Profiling

Activating Enzymes
Conjugating Enzymes
Ligases
Deconjugating Enzymes

Target/Substrate Proteins

Activating Enzymes
NF- κ B and IKK α
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

Ubiquitylation 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. Ubiquitylation has emerged as a critically important post-translational 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 fundamental types: that associated with the incorporation or removal of a functional group and that associated with the introduction of a functional protein (Table 1).

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.

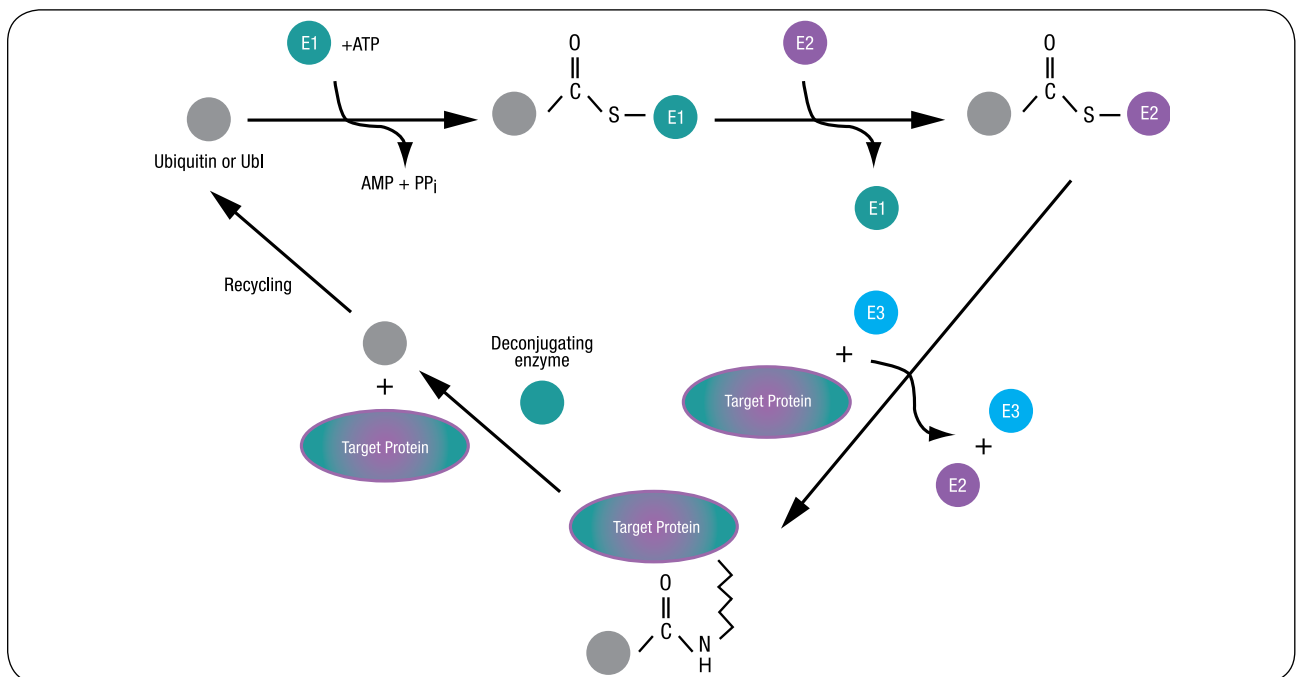
Ubiquitin is the 'parent' of a family of ubiquitin-like proteins (Ubls),

| Functional group/entity | Functional protein |
|----------------------------------|--------------------|
| Phosphate (-PO ₃ H) | Ubiquitin |
| Acetyl (Ac-/CH ₃ CO-) | SUMO-1, 2, 3 |
| Methyl (Me-/CH ₃) | NEDD8 |
| Sulphate (-SO ₃ 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 ubiquitinated substrates | 1 mg |
| Ubiquitin (human), (recombinant) (GST-tag) | BML-UW8620 | Studying ubiquitination <i>in vitro</i> and detection and purification of ubiquitinated substrates | 1 mg |
| Ubiquitin (human), (recombinant) | BML-UW0280 | Use in ubiquitination studies. | 1 mg |
| Ubiquitin (human), (fluorescein labeled) | BML-UW1240 | Use in ubiquitination studies. | 100 µg |
| Ubiquitin ⁺¹ , (recombinant) (His-tag) | BML-UW8790 | Recombinant frame-shift extended protein | 100 µg |
| Ubiquitin ⁵⁺¹ , (recombinant) (His-tag) | BML-UW8855 | Polyubiquitinated Ub ⁺¹ | 25 µg |
| [D ⁷⁷]Ubiquitin (human), (recombinant) (untagged) | BML-UW0345 | Incapable of C-terminal isopeptide bond formation | 1 mg |
| K ⁶ -only Ubiquitin (human), (recombinant) (untagged) | BML-UW0210 | Production of poly-ubiquitin chains via Lys ⁶ only | 1 mg |
| K ¹¹ -only Ubiquitin (human), (recombinant) (untagged) | BML-UW0215 | Production of poly-ubiquitin chains via Lys ¹¹ only | 1 mg |
| K ²⁷ -only Ubiquitin (human), (recombinant) (untagged) | BML-UW0220 | Production of poly-ubiquitin chains via Lys ²⁷ only | 1 mg |
| K ²⁹ -only Ubiquitin (human), (recombinant) (untagged) | BML-UW0225 | Production of poly-ubiquitin chains via Lys ²⁹ only | 1 mg |
| K ³³ -only Ubiquitin (human), (recombinant) (untagged) | BML-UW0230 | Production of poly-ubiquitin chains via Lys ³³ only | 1 mg |
| K ⁴⁸ -only Ubiquitin (human), (recombinant) (untagged) | BML-UW0235 | Production of poly-ubiquitin chains via Lys ⁴⁸ only | 1 mg |
| K ⁶³ -only Ubiquitin (human), (recombinant) (untagged) | BML-UW0240 | Production of poly-ubiquitin chains via Lys ⁶³ only | 1 mg |
| Ubiquitin, (biotinylated) | BML-UW8705 | Detection and purification of ubiquitinated substrates | 100 µg |
| Ubiquitin (bovine), (native) (methylated) | BML-UW8555 | Incapable of forming poly-ubiquitin chains via lysine linkages | 1 mg |
| [(N ^Σ -biotinyl)Lys ⁶]Ubiquitin | BML-UW8470 | Detection and purification of ubiquitinated substrates | 100 µg |
| [(N ^Σ -biotinyl)Lys ⁶ , (N ^Σ -biotinyl)Lys ⁴⁸]Ubiquitin | BML-UW8475 | Detection and purification of ubiquitinated substrates | 100 µg |
| [(N ^Σ -biotinyl)Lys ⁶ , (N ^Σ -biotinyl)Lys ⁶³]Ubiquitin | BML-UW8480 | Detection and purification of ubiquitinated substrates | 100 µg |
| Ubiquitin aldehyde, (recombinant) | BML-UW8450 | Inhibitor of deubiquitinating enzymes (DUBs) | 50 µg |
| Ubiquitin-AMC | BML-SE211 | Fluorogenic substrate for deubiquitinating enzymes (DUBs) | 25 µg |
| Ubiquitin vinyl sulphone, (HA-tag) | BML-UW0155 | Covalent inhibitors for detection and identification of deubiquitinating enzymes (DUBs) | 25 µg |
| Ubiquitin vinyl methyl ester, (HA-tag) | BML-UW0880 | Covalent inhibitors for detection and identification of deubiquitinating enzymes (DUBs) | 25 µg |
| Ubiquitin-Rhodamine | BML-SE761 | Substrate for deubiquitinating 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.^[1]

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 ¹ R]SUMO-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 |
| SUMO-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 SUMO-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*^[2,3] with mechanisms for formation proposed^[4].

[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^[5], although its role in certain disease states such as malignant transformation has recently been proposed^[6].

[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^[7]. 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^[8]. Again, as with ISG15 modification, a role in malignant transformation has been proposed^[9].

[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 |
| Ubl5 (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 ⁹³ R]SUMO-1 (human), (recombinant) (GST-tag) | BML-UW0175 | Use in proteomic studies | 100 µg |
| [K ¹¹ R]SUMO-2 (human), (recombinant) (GST-tag) | BML-UW0380 | Incapable of forming SUMO-2 chains at Lys ¹¹ | 100 µg |
| [K ¹¹ R]SUMO-3 (human), (recombinant) (GST-tag) | BML-UW0385 | Incapable of forming SUMO-3 chains at Lys ¹¹ | 100 µg |
| [K ¹¹ R]SUMO-3 (human), (recombinant) (GST-tag) | BML-UW0520 | Incapable of forming SUMO-3 chains at Lys ¹¹ | 100 µg |
| [K ¹¹ R]Ubiquitin (human), (recombinant) (untagged) | BML-UW0250 | Production of poly-ubiquitin chains via all lysines except Lys ¹¹ | 1 mg |
| [K ²⁷ R]Ubiquitin (human), (recombinant) (untagged) | BML-UW0255 | Production of poly-ubiquitin chains via all lysines except Lys ²⁷ | 1 mg |
| [K ²⁹ R]Ubiquitin (human), (recombinant) (untagged) | BML-UW0260 | Production of poly-ubiquitin chains via all lysines except Lys ²⁹ | 1 mg |
| [K ³³ R]Ubiquitin (human), (recombinant) (untagged) | BML-UW0265 | Production of poly-ubiquitin chains via all lysines except Lys ³³ | 1 mg |
| [K ⁴⁸ R]Ubiquitin (human), (recombinant) (untagged) | BML-UW8615 | Production of poly-ubiquitin chains via all lysines except Lys ⁴⁸ | 1 mg |
| [K ⁶³ R]Ubiquitin (human), (recombinant) (untagged) | BML-UW0275 | Production of poly-ubiquitin chains via all lysines except Lys ⁶³ | 1 mg |
| [K ⁶ R]Ubiquitin (human), (recombinant) (untagged) | BML-UW0245 | Production of poly-ubiquitin chains via all lysines except Lys ⁶ | 1 mg |
| [K ^{all} 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^α-amino group as well as on lysine N^ε-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^α- or N^ε-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 ^ε -biotinyl-Lys ⁶] | BML-UW8470 | Detection and purification of ubiquitinated substrates | 100 µg |
| Ubiquitin, [N ^ε -biotinyl-Lys ⁶ , N ^ε -biotinyl-Lys ⁴⁸] | BML-UW8475 | Detection and purification of ubiquitinated substrates | 100 µg |
| Ubiquitin, [N ^ε -biotinyl-Lys ⁶ , N ^ε -biotinyl-Lys ⁶³] | BML-UW8480 | Detection and purification of ubiquitinated substrates | 100 µg |
| Ubiquitin, biotinylated (randomly) | BML-UW8705 | Detection and purification of ubiquitinated substrates | 100 µg |
| Ubiquitin, methylated | BML-UW8555 | Incapable of forming poly-ubiquitin chains <i>via</i> lysine linkages | 1 mg |
| SUMO-1, biotinylated (randomly) | BML-UW0545 | Detection and purification of SUMOylated substrates | 100 µg |
| SUMO-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 |

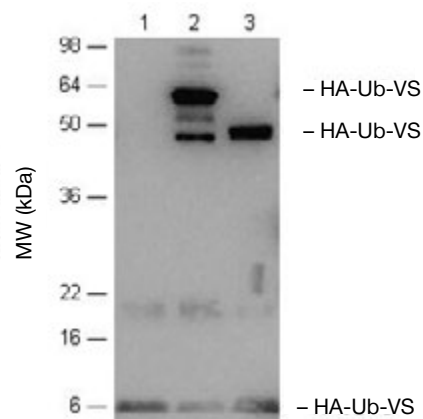
Ubiquitin & Ubl C-terminal and Side Chain Derivatives

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

HA-Ubiquitin Vinyl Sulphone

For the detection and identification of deubiquitinating enzymes, HA-Ub-VS is 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 |
| SUMO-1 [93-97]-AMCA | BML-UW0500 | Fluorogenic substrate for deSUMOylating enzymes | 1 mg |
| SUMO-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 deubiquitinating enzyme assays, and for polyubiquitin binding studies. Amongst other applications, the novel single isopeptide linkage-based polyubiquitinated 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^[10].

[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 | Substrates for deubiquitinating enzyme assays and polyubiquitin binding studies | 100 µg |
| ([K ⁶ only]Ub)n-ubiquitinated substrate | BML-UW0615 | | 25 µg |
| ([K ¹¹ only]Ub)n-ubiquitinated substrate | BML-UW0620 | | 25 µg |
| ([K ²⁷ only]Ub)n-ubiquitinated substrate | BML-UW0625 | | 25 µg |
| ([K ²⁹ only]Ub)n-ubiquitinated substrate | BML-UW0630 | | 25 µg |
| ([K ³³ only]Ub)n-ubiquitinated substrate | BML-UW0635 | | 25 µg |
| ([K ⁴⁸ only]Ub)n-ubiquitinated substrate | BML-UW0640 | | 25 µg |
| ([K ⁶³ only]Ub)n-ubiquitinated substrate | BML-UW0645 | | 25 µg |
| Di-ubiquitin (K ⁴⁸ -linked) | BML-UW9800 | | 100 µg |
| Di-ubiquitin (K ⁶³ -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 ₂₋₁₆) (K ⁴⁸ -linked) | BML-UW0670 | Substrates for deubiquitinating enzyme assays and polyubiquitin binding studies | 100 µg |
| Poly-ubiquitin chains (Ub ₂₋₇) (K ⁶³ -linked) | BML-UW9570 | | 100 µg |
| Polyubiquitin chains (Ub ₂₋₇), (linear) (recombinant) | BML-UW1010 | | 100 µg |
| Tetra-ubiquitin (K ⁴⁸ -linked) | BML-UW8645 | | 25 µg |
| Tetra-ubiquitin (linear) | BML-UW0785 | | 100 µg |
| Tri-ubiquitin (linear) | BML-UW0780 | | 100 µg |
| Ubn-ubiquitinated substrate | BML-UW0610 | | 25 µg |
| Undeca-ubiquitin (linear) | BML-UW0820 | | 100 µg |
| Poly-SUMO-3 chains ([SUMO-3] ₂₋₇) | BML-UW9675 | | PolySUMOylation studies |
| Poly-SUMO-2 chains ([SUMO-2] ₂₋₇) | 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 Ubl 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 polyubiquitinated species and, when used in concert, are capable of discriminating between these modification types. The introduction of the K⁶³-linkage specific monoclonal antibody BML-PW0600 [clone HWA4C4]^[11] signalled the very first commercially available ubiquitin isopeptide-linkage specific reagent. Such immunological tools are of huge value in the determination of ubiquitylation 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 polyubiquitinated conjugates monoclonal antibody (FK2) | BML-PW8810 | Species independent | IHC, WB | 500 µg |
| Mono- and polyubiquitinated conjugates monoclonal antibody (FK2) (ATTO 488 conjugate) | BML-PW1335 | Species independent | ICC | 25 µL |
| Mono- and polyubiquitinated conjugates monoclonal antibody (FK2) (biotin conjugate) | BML-PW0755 | Species independent | WB | 25 µL |
| Mono- and polyubiquitinated conjugates monoclonal antibody (FK2) (fluorescein labeled) | BML-PW1210 | Species independent | ICC | 25 µL |
| Mono- and polyubiquitinated conjugates monoclonal antibody (FK2) (HRP conjugate) | BML-PW0150 | Wide range of species | ELISA, IHC, WB | 25 µg / 100 µg |
| Polyubiquitin (K ⁶³ -linkage-specific) monoclonal antibody (clone HWA4C4) (HRP conjugate) | BML-PW0605 | Wide range of species | WB | 25 µg / 100 µg |
| Polyubiquitin (K ⁶³ -linkage-specific) monoclonal antibody (HWA4C4) | BML-PW0600 | Wide range of species | ELISA, IHC, ICC, WB | 25 µg / 100 µg |
| Polyubiquitinated conjugates monoclonal antibody (FK1) | BML-PW8805 | Species independent | IHC, WB | 500 µg |
| Ub ⁺¹ polyclonal antibody | BML-PW9780 | Human | WB | 25 µg / 100 µg |

Ubiquitin-reactive Antibodies

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

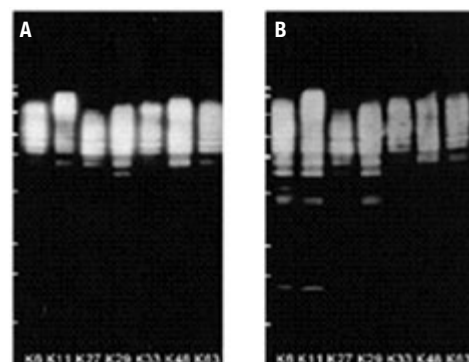


Figure 2: Immunodetection of single lysine-linked polyubiquitin chains by western blotting following SDS-PAGE using [A] BML-PW8805 (clone FK1) and [B] BML-PW8810 (clone FK2).

| 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 K⁶³-linked polyubiquitin chains is implicated in a variety of cellular events, including DNA repair, signal transduction and receptor endocytosis. BML-PW0600 specifically recognizes K⁶³-linked polyubiquitin, but NOT any other isopeptide-linked (K⁶, K¹¹, K²⁷, K²⁹, K³³, or K⁴⁸) polyubiquitinated species. This unique monoclonal antibody is a powerful tool facilitating the analysis of K⁶³-linked polyubiquitylation.

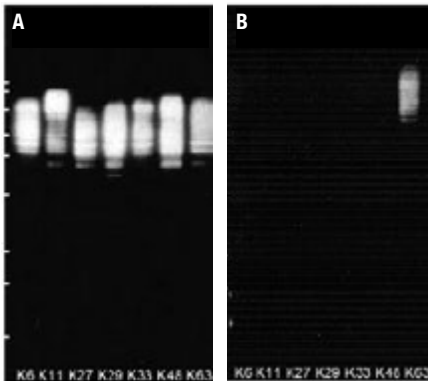


Figure 3: Western blot following SDS-PAGE of single lysine mutant chains probed with pan-reactive mAb FK1 [A] (BML-PW8805) & K⁶³-linkage specific mAb HWA4C4 [B] (BML-PW0600).

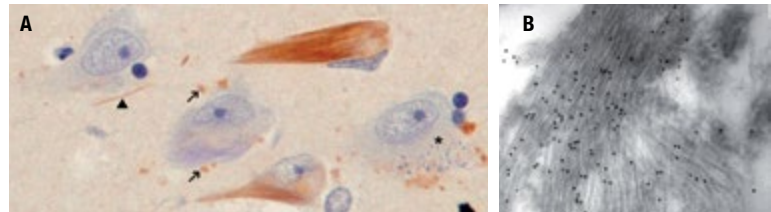


Figure 4: [A] Sections through the hippocampus in Alzheimer's Disease (AD), stained using mAb HWA4C4 (BML-PW0600) and showing differential staining of neurofibrillary tangles (NFTs). [B] Immunogold labelling TEM in AD using HWA4C4 mAb (BML-PW0600) provides evidence that K⁶³-linked polyubiquitin is present in NFTs [11].

| 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 |
| Ubl5 (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 # | Utility | Size |
|---|------------|--|--------|
| 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) polyclonal 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 ⁸⁵ A]UbcH5a (human), (recombinant) (His-tag) | BML-UW9055 | 100 µg |
| [C ⁸⁵ 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 |
| RanBP2ΔFG (human), (recombinant) (GST-tag) | BML-UW9455 | 100 µg |

Ligases (E3s) – Antibodies

| Product Name | Product # | Specificity | Application | Size |
|---|---------------|---|-------------|----------------|
| AtCul3 polyclonal antibody | BML-PW0470 | <i>Arabidopsis</i> | IP, WB | 25 µL |
| AtRbx1 polyclonal antibody | BML-PW0465 | <i>Arabidopsis</i> | 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 polyubiquitinated 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 deubiquitinating 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^[12].

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 deubiquitinate. 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 deubiquitination arises from the association of DUBs with these protein partners^[13].

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/Ubl-related diseases.

[12] Deubiquitinating 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 | Ubiquitin-specific proteases | 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 | | 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 | | NEDD8-specific proteases |
| NEDP1 (human), (recombinant) | ALX-201-171 | 50 µg | |
| NEDP1 (human), (recombinant) (His-tag) | BML-UW9770 | 100 µg | |
| SEN2 (catalytic domain) (human), (recombinant) (GST-tag) | BML-UW9765 | 100 µg | |
| Otubain-1 (human), (recombinant) (His-tag) | BML-UW0680 | SUMO-specific proteases | 50 µg |
| SEN1 (catalytic domain) (human), (recombinant) (GST-tag) | BML-UW9760 | | 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 ¹¹ only]Ub)n-ubiquitinated substrate | BML-UW0620 | Substrates for deubiquitinating enzyme assays and polyubiquitin binding studies | 25 µg |
| ([K ²⁷ only]Ub)n-ubiquitinated substrate | BML-UW0625 | | 25 µg |
| ([K ²⁹ only]Ub)n-ubiquitinated substrate | BML-UW0630 | | 25 µg |
| ([K ³³ only]Ub)n-ubiquitinated substrate | BML-UW0635 | | 25 µg |
| ([K ⁴⁸ only]Ub)n-ubiquitinated substrate | BML-UW0640 | | 25 µg |
| ([K ⁶ only]Ub)n-ubiquitinated substrate | BML-UW0615 | | 25 µg |
| ([K ⁶³ only]Ub)n-ubiquitinated substrate | BML-UW0645 | | 25 µg |
| Di-ubiquitin (K ⁴⁸ -linked) | BML-UW9800 | | 100 µg |
| Di-ubiquitin (K ⁶³ -linked) | BML-UW0730 | | 50 µg |
| Linear polyubiquitin chains, sampler pack | BML-UW0825 | | 1 Pack |
| Poly-ubiquitin chains (Ub ₂₋₁₆) (K ⁴⁸ -linked) | BML-UW0670 | | 100 µg |
| Poly-ubiquitin chains (Ub ₂₋₇) (K ⁶³ -linked) | BML-UW9570 | | 100 µg |
| Tetra-ubiquitin (K ⁴⁸ -linked) | BML-UW8645 | | 25 µg |
| Ubn-ubiquitinated substrate | BML-UW0610 | | 25 µg |
| TCID | BML-EI399 | UCH-L3 inhibitor | 10 mg / 50 mg |
| Ubiquitin-AMC | BML-SE211 | Fluorogenic substrate for deubiquitinating enzymes (DUBs) | 25 µg |
| Ubiquitin aldehyde, (recombinant) | BML-UW8450 | Inhibitor of deubiquitinating enzymes (DUBs) | 50 µg |
| Ubiquitin ⁵⁺¹ , (recombinant) (His-tag) | BML-UW8855 | Polyubiquitinated Ub ⁺¹ | 25 µg |
| Ubiquitin vinyl methyl ester, (HA-tag) | BML-UW0880 | Covalent inhibitors for detection and identification of deubiquitinating enzymes (DUBs) | 25 µg |
| Ubiquitin vinyl sulphone, (HA-tag) | BML-UW0155 | | 25 µg |
| Z-Leu-Arg-Gly-Gly-AMC | BML-P801 | Fluorogenic substrates for deubiquitinating enzymes (DUBs) | 5 mg |
| Z-Arg-Leu-Arg-Gly-Gly-AMC | BML-ZW8585 | | 5 mg |
| SUMO | | | |
| SUMO-2 aldehyde | BML-UW0065 | Specific inhibitors of deSUMOylating enzymes | 25 µg |
| SUMO-1-AMC | BML-UW0040 | Fluorogenic substrates for deSUMOylating enzymes | 50 µg |
| SUMO-1 (93-97)-AMCA | BML-UW0500 | | 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 |
| SEN2 (mouse) polyclonal antibody | ALX-210-482 | Mouse | ELISA, WB | 100 µg |
| SEN2 polyclonal antibody | ALX-210-863 | Human, Mouse | WB | 200 µL |
| SEN5 (human) polyclonal antibody | BML-PW0365 | Human | WB | 25 µL / 100 µL |
| SEN5 polyclonal antibody | ALX-210-865 | Human, Mouse | WB | 200 µL |
| SEN6 (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^[14-16]. PGP9.5 is a member of the ubiquitin C-terminal hydrolase family^[17,18] and immunohistochemical studies have shown that the protein is enriched in several ubiquitinated inclusion bodies, suggesting that such structures may be metabolically dynamic regions of the cell^[19]. The antiserum may be used in Western blotting (24 kDa)^[20] 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.

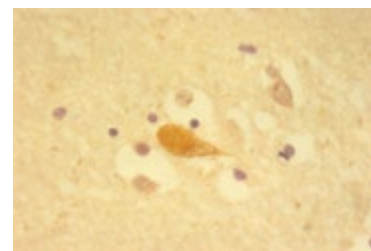


Figure 5: Cortical Lewy body in human brain immunostained using the rabbit antiserum to PGP9.5 (BML-PG9500). Micrograph courtesy of Prof. RJ Mayer (University of Nottingham).

[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- κ 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- κ 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- κ B factors are retained in an inactive state by binding to the inhibitor of NF- κ B (I κ B) which, in response to cell stimulation, is ubiquitinated (by derivatization with K⁴⁸-linked chains) and degraded by the proteasome^[21]. Prior to its ubiquitination, I κ B is phosphorylated by the I κ B kinase (IKK) complex. The IKK complex, consisting of two kinases IKK α and IKK β , and the regulatory component NEMO, is activated by an upstream kinase (TAK1) which is in turn activated after TNF α or IL-1 receptor stimulation^[22].

[21] Ubiquitin signals in the NF-kappaB pathway: J. Terzic, *et al.*; Biochem. Soc. Trans. 35, 942 (2007)

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

NF- κ B and IKK α – Proteins

| Product Name | Product # | Detail / Use | Size |
|---|-------------|---|------------|
| I κ B α (human), (recombinant) (GST-tag) | BML-UW9970 | Ubiquitinylation/SUMOylation substrates | 50 μ g |
| I κ B α (human), (recombinant) (untagged) | BML-UW9975 | | 50 μ g |
| NF- κ B (p50) (human), (recombinant) (His-tag) | ALX-201-285 | | 2 μ g |
| NF- κ B p50 subunit (human) (35-381), (recombinant) (GST-tag) | BML-UW9980 | | 50 μ g |
| NF- κ B p50 subunit (human) (35-381), (recombinant) (untagged) | BML-UW9985 | | 50 μ g |
| NF- κ B (p65) (human), (recombinant) (His-tag) | ALX-201-284 | | 2 μ g |
| NF- κ B p65 subunit (human) (12-317), (recombinant) (GST-tag) | BML-UW9990 | | 50 μ g |
| NF- κ B p65 subunit (human) (12-317), (recombinant) (untagged) | BML-UW9995 | | 50 μ g |

NF- κ B and IKK α – Antibodies

| Product Name | Product # | Specificity | Application | Size |
|---|---------------|---|-----------------|--------------------------|
| I κ B α monoclonal antibody (6A920) | ALX-804-209 | Human, Mouse | FC, IHC, IP, WB | 100 μ g |
| [pSer32/36]I κ B α polyclonal antibody | BML-SA412 | Human, Mouse, Porcine | WB | 20 μ L / 100 μ L |
| [pSer32/Ser36]I κ B α monoclonal antibody (39A1413) | ADI-KAM-TF140 | Human, Mouse, Rat, Bovine, Dog, Porcine | IP, WB | 100 μ g |

IKK α – Substrate

| Product Name | Product # | Detail / Use | Size |
|--|-----------|--|------|
| I κ 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^[23]. 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 | Ubiquitinylation/SUMOylation substrates | 20 µg |
| p53 (human), (recombinant) (GST-tag) | BML-FW9370 | | 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^[24]. 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 (<i>Arabidopsis thaliana</i>) polyclonal antibody | BML-PW0190 | <i>Arabidopsis thaliana</i> | 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 polyubiquitinated 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 | Protein interaction studies | 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 | | 0.5 mL |
| SUMO-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-ubiquitylation 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^[25].

[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), (recombinant) (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 Ψ-K-X-D/E (where lysine is the amino acid modified, Ψ 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

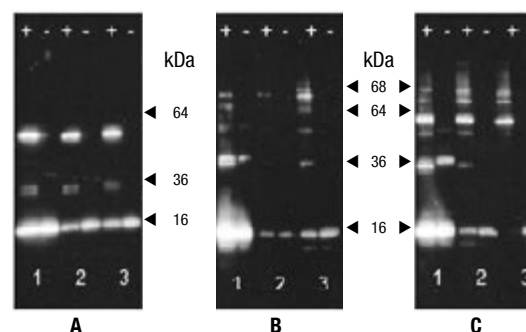


Figure 6: Western blots following SDS-PAGE of SUMOylation assays using: [A] RANGAP1 (BML-UW9755); [B] SP100 (BML-UW9825); and [C] p53 (BML-FW9370) as substrate proteins with the three SUMOs assayed in the presence (+) and absence (-) of ATP—lane 1: SUMO-1 (BML-UW9195); lane 2: SUMO-2 (BML-UW9205); and 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® Kit was specifically developed for the isolation and enrichment of ubiquitinated proteins. The kit facilitates the isolation of both mono- and poly-ubiquitinated 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® 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 ubiquitinated protein conjugates from a specific cell/tissue lysate
- Capture and analysis of specific ubiquitinated protein conjugates of interest from particular cell/tissue lysates
- Separation of ubiquitinated/non-ubiquitinated 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® matrix using a deubiquitinating enzyme
- Release of ubiquitinated proteins in their active/native form by elution from the UBI-QAPTURE-Q® matrix using high salt buffer

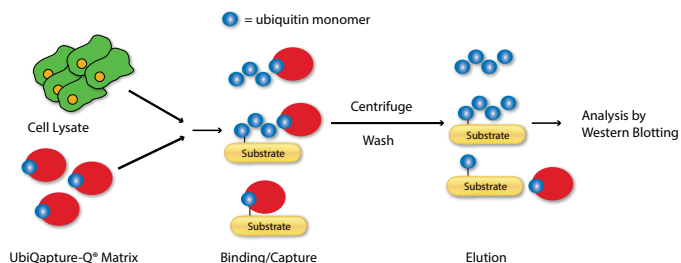
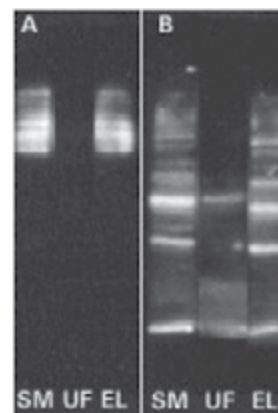


Figure 7: Schematic overview of UbiQapture Kit isolation and detection process.

Figure 8: Western blot analysis demonstrating ubiquitin enrichment of partially purified and lysate-derived ubiquitinated proteins after UBI-QAPTURE-Q®. 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 ubiquitinated Ubch5a from *in vitro* ubiquitinylation assay. B: Capture of Ub-protein conjugates from control ubiquitinated-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 experiments | 1 mg |
| Fraction II (HeLa) | BML-HW8605 | | 1 mg |
| HeLa S100 fraction | BML-SW8750 | For demonstrating ubiquitin-proteasome mediated conjugation/degradation | 1 mg |
| Mg ²⁺ /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 α subunit (human), (recombinant) (GST-tag) | BML-PW9120 | 100 μ g |
| Proteasome 11S β subunit (human), (recombinant) (GST-tag) | BML-PW9125 | 100 μ g |
| Proteasome 11S γ subunit (human), (recombinant) (GST-tag) | BML-PW9130 | 100 μ g |
| Proteasome activator 11S complex (human), (purified) | BML-PW9420 | 25 μ g |
| Proteasome activator 11S α subunit (human), (recombinant) | BML-PW9865 | 100 μ g |
| Proteasome activator 11S β subunit (human), (recombinant) | BML-PW9870 | 100 μ g |
| Proteasome activator 11S γ subunit (human), (recombinant) | BML-PW9875 | 100 μ g |

11S Activator – Antibodies

| Product Name | Product # | Specificity | Application | Size |
|---|------------|-------------------|-------------|--------------------------|
| Proteasome activator 11S α subunit polyclonal antibody | BML-PW8185 | Human, Mouse, Rat | IHC, WB | 25 μ L / 100 μ L |
| Proteasome activator 11S β subunit polyclonal antibody | BML-PW8240 | Human, Mouse, Rat | IHC, WB | 25 μ L / 100 μ L |
| Proteasome activator 11S γ subunit polyclonal antibody | BML-PW8190 | Human, Mouse | IHC, WB | 25 μ L / 100 μ L |
| Proteasome activator 11S subunit antibody sampler pack | BML-PW8915 | Human, Mouse | IHC, WB | 3 x 10 μ L |

Miscellaneous Activator Complexes – Antibodies

| Product Name | Product # | Specificity | Application | Size |
|---|------------|---------------------------------|-------------|--------------------------|
| Blm10 (<i>Saccharomyces cerevisiae</i>) polyclonal antibody | BML-PW0570 | <i>Saccharomyces cerevisiae</i> | WB | 25 μ L |
| POMP polyclonal antibody | BML-PW9715 | Human, Rat, Monkey | IHC, IP, WB | 25 μ L / 100 μ L |

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 | <i>Arabidopsis</i> , 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 ₆ -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 | Human | IP | 25 µL / 100 µL |
| Proteasome 19S Rpn12/S14 subunit (human) monoclonal antibody (P31-38) | BML-PW9260 | Human | WB | 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 | <i>Arabidopsis</i> , 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 | <i>S. Cerevisiae</i> , <i>A. Thaliana</i> | WB | 25 µL |
| Proteasome 19S Rpn6/S9 subunit polyclonal antibody | BML-PW8370 | <i>Arabidopsis</i> , 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 (<i>Saccharomyces cerevisiae</i>) | BML-PW8775 | Purified from <i>Saccharomyces cerevisiae</i> | 50 µg |

Proteasome-associated Proteins – Antibodies

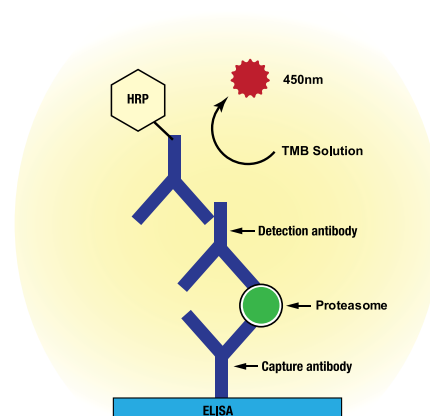
| Product Name | Product # | Specificity | Application | Size |
|--|-------------|--------------------|-------------|----------------|
| BAG-1 (mouse) (CT) polyclonal antibody (Bur 1702) | ALX-210-009 | Mouse | IHC, IP, WB | 50 µL |
| PAC1 (human) monoclonal antibody (EX-5) | BML-PW0480 | Human | IP, 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 (<i>Arabidopsis thaliana</i>) polyclonal antibody | BML-PW0430 | <i>Arabidopsis</i> | WB | 25 µL / 100 µL |
| PBF1 (<i>Arabidopsis thaliana</i>) polyclonal antibody | BML-PW0435 | <i>Arabidopsis</i> | WB | 25 µL / 100 µL |
| PI31 polyclonal antibody | BML-PW9710 | Human, Mouse | WB | 25 µL / 100 µL |

Proteasome 20S Assay Kits

Proteasome ELISA Kit (BML-PW0575)

Proteasomes are non-lysosomal proteolytic complexes localized primarily in the cytoplasm and in the nucleus of eukaryotic cells^[26]. 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^[27-29].

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.



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

[26] Immunological methods to quantify and characterize proteasome complexes: development and application: Majetschak & L. T. Sorell; J. Immunol. Methods. 334, 91-103 (2008)

[27] Serum concentration and localization in tumor cells of proteasomes in patients with hematologic malignancy and their pathophysiologic significance: M. Wada, *et al.*; J. Lab. Clin. Med. 121, 215-223 (1993)

[28] Circulating proteasomes are markers of cell damage and immunologic activity in autoimmune diseases: K. Egerer, *et al.*; J. Rheumatol. 29, 2045-2052 (2002)

[29] Circulating proteasome levels are an independent prognostic factor for survival in multiple myeloma: C. Jakob, *et al.*; Blood. 109, 2100-2105 (2007)

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

Proteasome 20S β -Subunits – Antibodies

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

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-Nle-CHO | • | | • | BML-P120 | 5 mg / 25 mg |
| Aclacinomycin A (Aclarubicin) | • | | | BML-AW8655 | 5 mg |
| Ada-(Ahx) ₃ -(Leu) ₃ -vinyl sulfone | • | | • | BML-AW9155 | 100 µg |
| Ada-Tyr-(Ahx) ₃ -(Leu) ₃ -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 β-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-Leu-B(OH) ₂ (MG262) | • | | | BML-PI109 | 100 µg |
| Z-Leu-Leu-Leu-H (MG132) | • | | | BML-PI102 | 5 mg / 25 mg |
| Z-Leu-Leu-Leu-vinyl sulfone | • | • | | BML-ZW9170 | 500 µg |
| Z-Leu-Leu-Tyr-ketoaldehyde | • | | | BML-ZW8655 | 5 mg |
| Z-Pro-Nle-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-Nle-Pro-Nle-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 ubiquitinated 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 ubiquitinated protein substrates. The preparation consists of a high purity mixture of '26S' proteasomes singly (26S) and doubly (30S) capped with 19S regulatory 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 | <i>Arabidopsis</i> , 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 | Use in general and selective autophagy studies | 500 µg |
| GABARAP-L2 (human), (recombinant) (GST-tag) | BML-UW1195 | | 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 ubiquitinated 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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