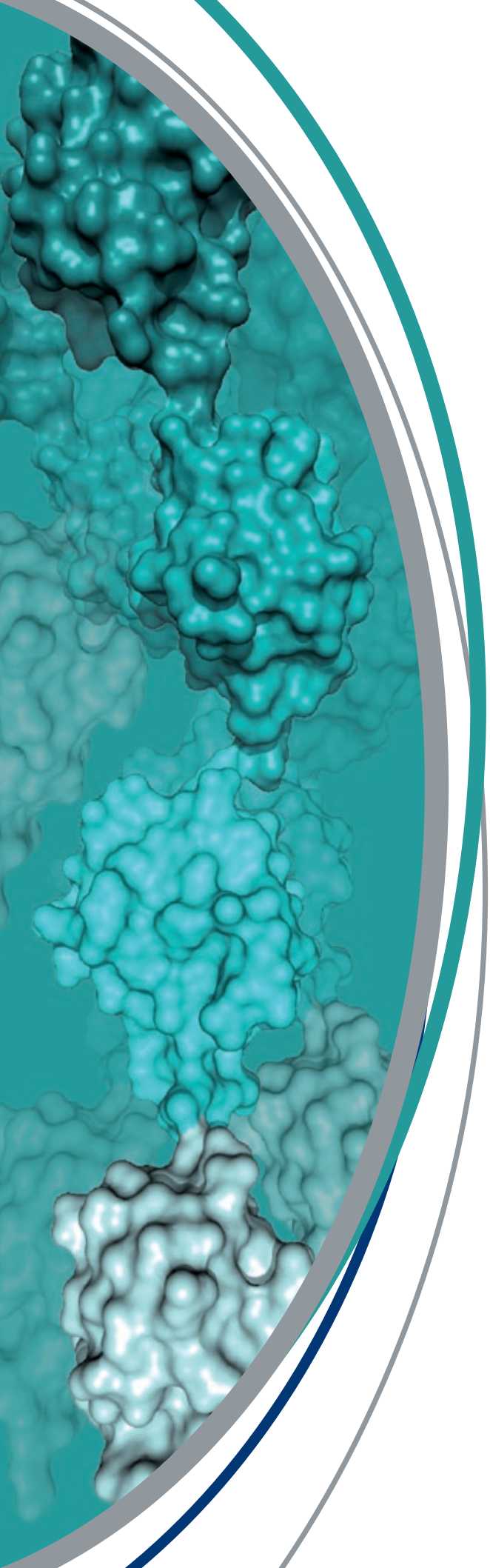

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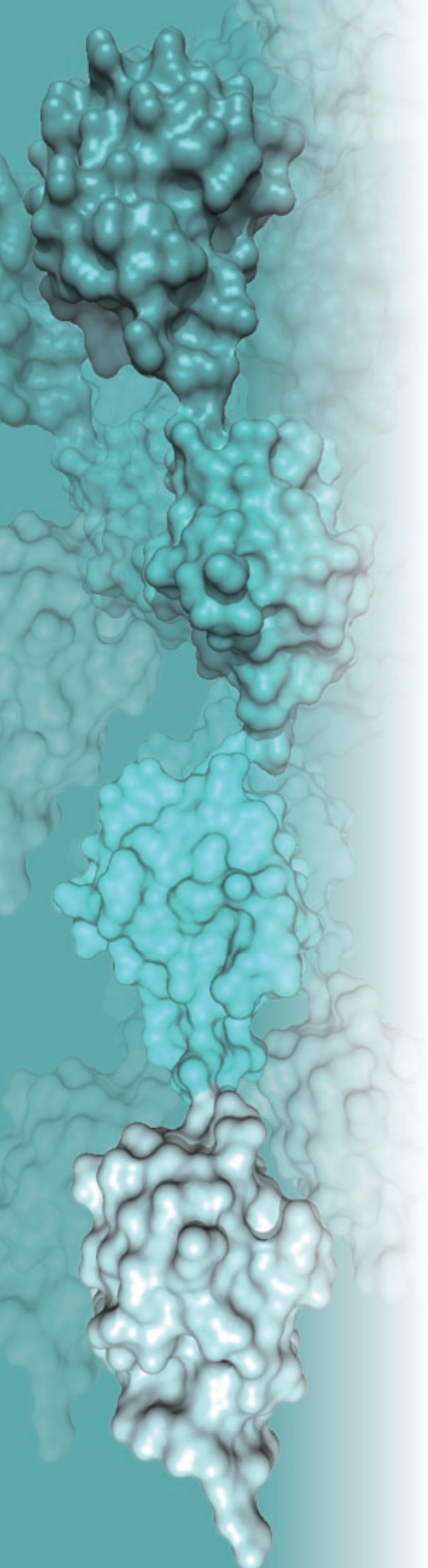

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International Edition 09/2009

Content

Key to Abbreviations

Proteins
Human, recombinant, untagged unless stated otherwise

Polyclonal Antibodies
Rabbit pAb unless stated otherwise

Monoclonal Antibodies
Mouse mAb unless stated otherwise

Purified (PF) = Purified (Preservative free); FC = Flow Cytometry; ICC = Immunocytochemistry; IP = Immunoprecipitation; IHC = Immunohistochemistry (FS = Frozen Sections, PS = Paraffin Sections); WB = Western blot; BP = Blocking Peptide

COVER IMAGE: K^{63}-linked tetraubiquitin. *Courtesy of Dr David Komander, MRC-LMB, Cambridge, UK*

Overview

Ubiquitinylation of cellular proteins is a highly complex, temporally controlled, and tightly regulated process that targets, in a specific manner, thousands of cellular proteins. It is carried out by a modular cascade of enzymes with high specificity towards target proteins. Ubiquitinylation 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 first description in 1975 [1] it has been apparent that ubiquitin has a fundamental importance in cellular biochemistry. A small protein of only seventy six amino acids and a molecular weight of ~8.6kDa, 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 of protein post-translational modifications, signaling for many differing cellular events, and being closely inter-linked with other post-translational modifications such as phosphorylation and acetylation.

Functional group/entity	Functional protein
Phosphate (-PO_3H)	Ubiquitin
Acetyl (Ac-/CH_3CO-)	SUMO-1, 2, 3
Methyl (Me-/CH_3-)	NEDD8
Sulphate (-SO_3H)	ISG15
Lipid	FAT10
Carbohydrate	Urm1

TABLE 1: Types of post-translational modification

Ubiquitin is the 'parent' of a family of ubiquitin-like proteins (Ubls) of which at least ten members are currently identified. At the amino acid level the homology amongst these 'family' members is low (see Table 2); however, it is not amino acid homology that forms the primary basis for family membership. In addition to similarity in their modes of action and functionality, the ubiquitin superfold [2] forms a structural component, almost identical to that of ubiquitin, that is shared amongst Ubl family members and which provides a stable scaffold on which different epitopes can mediate specific interactions with binding proteins & 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 [3].

	Ubiquitin	SUMO-1	SUMO-2	SUMO-3	NEDD8	ISG15a	ISG15b	FAT10a	FAT10b	Urm1	Ubl5	Fub1	Atg12	Atg8
Ubiquitin		18	16	16	58	29	37	29	36	12	22	37	17	10
SUMO-1			54	54	21	16	20	16	15	10	13	23	16	13
SUMO-2				100	18	16	13	8	11	11	15	19	10	13
SUMO-3					18	16	13	8	11	11	15	19	10	13
NEDD8						28	26	26	29	13	18	24	17	6
ISG15a							26	20	22	11	17	18	9	8
ISG5b								21	23	17	14	35	11	10
FAT10a									20	11	20	27	8	12
FAT10b										13	19	31	9	9
Urm1											9	10	3	9
Ubl5												14	10	11
Fub1													10	11
Atg12														26
Atg8														

TABLE 2: Ubiquitin/Ubl amino acid sequence homologies. Data courtesy of K Hoffman, Miltenyi Biotec.

Ubiquitin contains seven internal lysine residues and terminates with a C-terminal glycyl-glycine motif. It is through this C-terminal glycine residue that ubiquitin attaches itself to the ε-amino group of the side chain of lysine residues within substrate proteins *via* the formation of an isopeptide bond. In this way, ubiquitin may attach itself as a monomer (mono-ubiquitinylation), as a multiple monomer (multi-ubiquitinylation), or by internal extension as a polymer (poly-ubiquitinylation) (Figure 1). The fate of the modified substrate protein will depend upon the exact nature and extent of the modification.

Each of the seven lysine residues within ubiquitin (Figure 2) is capable of chain initiation and thus may give rise to one of seven different chain types, assuming that the integrity of chain type is conserved during chain extension. All seven chain types have been identified in mammalian cells *in vivo* [4] with matters being greatly complicated by the reporting of possible mixed and forked chain types [5]. If mixed chain types are found to be a common form of protein polyubiquitinylation with the subsequent fate of modified substrates being dependent upon the nature and ordering of the chain linkage types, then a new area of sequencing technology will be called for in order to decipher the whole spectrum of resultant ubiquitin chain structures.

The conformations of the polyubiquitin chains formed are dependent upon the nature of the isopeptide linkage (for an example see Figure 3). In addition to isopeptide-linked ubiquitin chains, evidence has also been provided for the existence of chains formed by linkages between the C- and N-termini of sequential ubiquitin subunits, thereby assembling a novel head-to-tail linear polyubiquitin chain. This linear polyubiquitin chain generated post-translationally may perform as yet another modulator of protein function [6].

Recognition that protein substrates may be modified with ubiquitin at sites other than lysine, namely cysteine, serine, and threonine residues, serves to complicate matters even further [7].

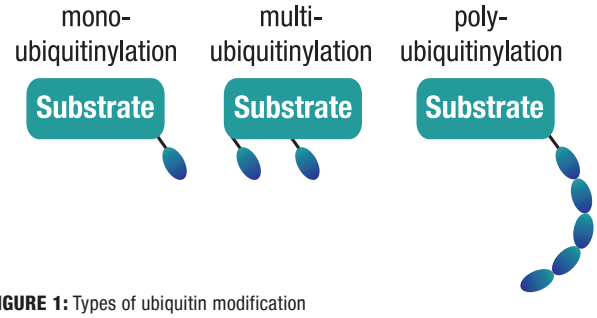

FIGURE 1: Types of ubiquitin modification

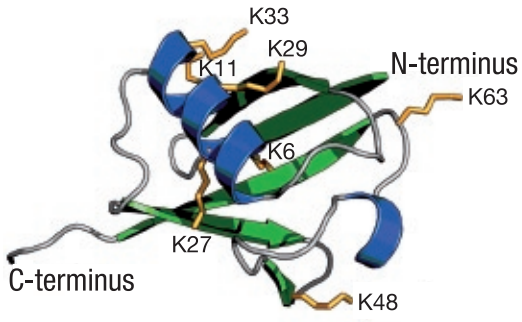

FIGURE 2: Lysine location within ubiquitin

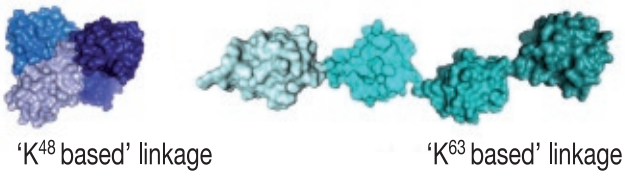

FIGURE 3: K^{48} -and K^{63}-linked tetra-ubiquitin. *Image courtesy of D. Komander, MRC LMB, Cambridge, UK*

At least ten different ubiquitin-like modifiers have been described in mammalian cells and conjugation of each modifier to its target may result in a different biological effect. In many cases proteins are modified by multiple moieties of ubiquitin (or SUMO or NEDD8) generating a polysubunit chain. Modification remodels the surface of the target proteins, affecting, among other properties, their stability, interactions with other proteins, activity, and subcellular localization. It is already recognized that particular modification states and ubiquitin linkage types predispose to a certain fate for the substrate molecule. For many proteins, modification with ubiquitin (*via* a K^{48}-linked polyubiquitin chain) leads to their degradation by the 26S proteasome. Yet, dependent on the nature of the isopeptide linkage between the ubiquitin moieties, it may also lead to other fates. Conjugation of ubiquitin or one of the ubiquitin-like proteins can serve a variety of non-proteolytic functions, including activation of enzymes, modulation of membrane dynamics, or routing of the tagged proteins to their sub-cellular destination (Figure 4); however, such classification is unlikely to be absolute or exclusive.

The attachment of ubiquitin to the ε-amino of lysine residues of target proteins requires a series of ATP-dependent enzymatic steps by ubiquitin activating (E1), ubiquitin conjugating (E2) and ubiquitin ligating (E3) enzymes. Consequently, protein ubiquitinylation is achieved through a minimum of three enzymatic steps (Figure 5). In the first step, in an ATP-dependent process, a ubiquitin-activating enzyme (E1) catalyz-

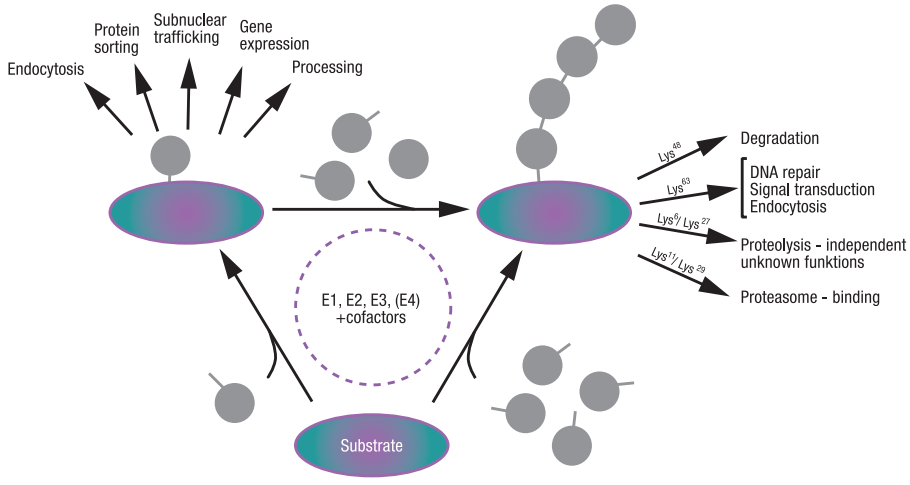

FIGURE 4: Differing ubiquitin modification resulting in distinct functions.

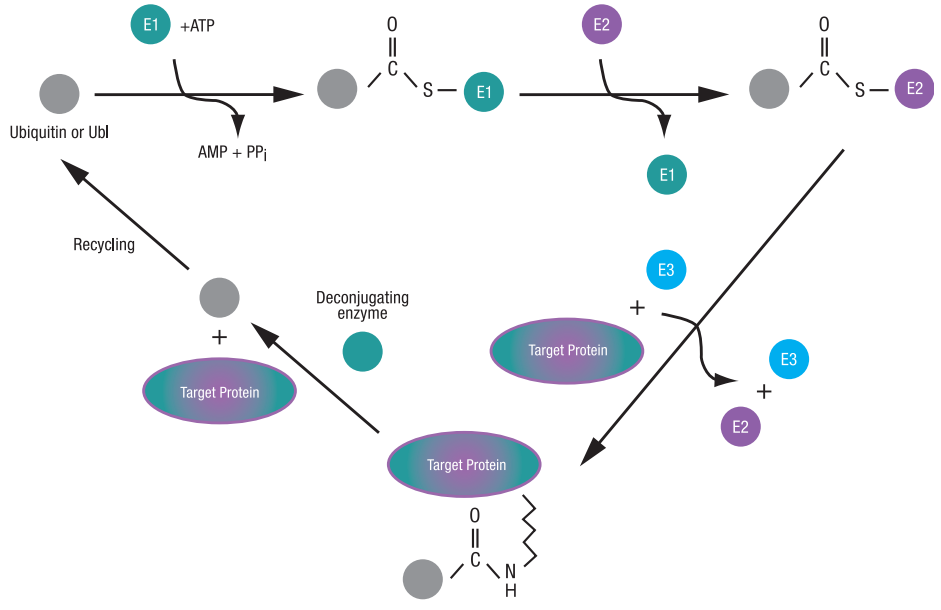

FIGURE 5: Ubiquitin cascade showing activation, conjugation, ligation, deconjugation, and recycling steps.

es the formation of a reactive thioester bond with ubiquitin. This is followed by its subsequent transfer to the active site cysteine of a ubiquitin carrier protein (E2). The specificity of ubiquitin ligation arises from the subsequent association of the E2-ubiquitin thioester with a substrate-specific ubiquitin-protein isopeptide ligase (E3), which facilitates the formation of the isopeptide linkage between ubiquitin and its target protein. In most cases, *i.e.* the RING finger domain E3s, the E3 serves as a scaffold that brings together the E2 and the substrate into proximity allowing for efficient transfer of the activated ubiquitin moiety from E2 to the substrate. In other cases, such as the HECT domain E3s, the activated ubiquitin is transferred from E2 to an internal Cys residue on E3 before conjugation of ubiquitin to the target substrate. Here, the E3 has a catalytic role. An additional subset of E3s (U-box domain), also termed E4s, serves as a scaffold to aid in the transfer of ubiquitin from the E2 to a previously conjugated ubiquitin moiety, in effect elongating polyubiquitin chains [review see 8].

Similar cascades are involved for the modification of substrate proteins with the various ubiquitin-like proteins [9]. The Ubls also function as critical regulators of many cellular processes, including transcription, DNA repair, signal transduction, autophagy, and cell-cycle control with a growing body of data implicating the dysregulation of Ubl-substrate modification and mutations in the Ubl-conjugation machinery in the etiology and progression of a number of human diseases.

There is increasing evidence for the concerted interaction and interplay between the various pathways; for example that of ubiquitin, SUMO and NEDD8 in NF-κB signaling [10] and between ubiquitin and autophagy-specific Ubls in selective autophagy [11].

Ubiquitin/Ubl-protein conjugates are highly dynamic structures. While an array of enzymes directs the conjugation of these modifiers to substrates, there are also dozens of deconjugating enzymes (DCEs) that can reverse the process. There is much evidence to indicate that DCEs are important regulators of the ubiquitin/Ubl systems. These enzymes are responsible for processing inactive precursors, proof-reading protein conjugates, removing ubiquitin/Ubl from cellular adducts, and keeping the 26S proteasome free of inhibitory ubiquitin chains [12]. The importance of various DCEs is now well established; however, a detailed understanding of both their selectivity and reactivity remains comparatively poor, not least due to the current lack of availability of high purity, full length, functionally competent enzymes together with appropriate substrates facilitating the dissection of selectivity and specificity of action.

The complexity of the ubiquitin and ubiquitin-like protein cascades is considerable. In mammals, there are at least two ubiquitin activating enzymes known, some twenty plus conjugating enzymes, over eight hundred ligases, and approaching one hundred deconjugating enzymes. These varied components work in a hierarchical context and for appropriate ubiquitinylation to occur the correct combination of E1, E2, E3, substrate, and deconjugating enzyme must all work in concert. The similar cascades for the ubiquitin-like proteins appear not to be as complex as that of ubiquitin with a reduced number of component possibilities.

As our knowledge of the pathways involved in the modification of substrates with ubiquitin/Ubls has grown, so have the ties between these modifications and human disease. To date aberrancy in the ubiquitin/Ubl signaling systems has been implicated in diseases as diverse as malignancies (cancer), hypertension, mental retardation, neurodegenerative disease, cystic fibrosis, immune system malfunction, inflammatory response, and muscle wasting. It is this essential involvement in cellular biochemistry and the development of disease that is driving the continued research effort at an academic level and the interest by the pharmaceutical and biotechnology industry from a drug discovery viewpoint.

The future challenge for Enzo Life Sciences is to continue its considerable efforts over the past decade and more and continue to develop and produce more robust tools that facilitate improved study and a greater understanding of the ubiquitin and ubiquitin-like protein pathways.

LITERATURE REFERENCES:

[1] The complete amino acid sequence of ubiquitin, an adenylate cyclase stimulating polypeptide probably universal in living cells: D.H. Schlesinger, et al.; Biochemistry **14,** 2214 (1975)
[2] Ubiquitin superfolds: intrinsic and attachable regulators of cellular activities?: R.J. Mayer, et al.; Fold. Des. **3,** R97 (1998)
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[5] Certain pairs of ubiquitin-conjugating enzymes (E2s) and ubiquitin-protein ligases (E3s) synthesize nondegradable forked ubiquitin chains containing all possible isopeptide linkages: H.T. Kim, et al.; J. Biol. Chem. **282,** 17375 (2007)
[6] A ubiquitin ligase complex assembles linear polyubiquitin chains: T. Kirisako, et al.; EMBO J. **25,** 4877 (2006)
[7] Ubiquitylation on canonical and non-canonical sites targets the transcription factor neurogenin for ubiquitin-mediated proteolysis: J.M. Vosper, et al.; J. Biol. Chem. **284,** 15458 (2009)
[8] The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction: M.H. Glickman & A. Ciechanover; Physiol. Rev. **82,** 373 (2002)
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Ubiquitin, Ubiquitin-like Proteins & their Derivatives

Ubiquitin is the founding member of a family of structurally conserved proteins, the ubiquitin-like proteins (Ubls), which include the members SUMO1, 2, & 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.

Ubiquitin

Product	Utility	Prod. No.	Size
Ubiquitin		BML-UW8795-0005	5 mg
Ubiquitin, agarose conjugate	For affinity purification of ubiquitin-binding proteins	BML-UW8630-0500	0.5 ml
Ubiquitin, His_6-tagged	For the detection and purification of ubiquitinylated substrates	BML-UW8610-0001	1 mg
Ubiquitin, GST-tagged		BML-UW8620-0001	1 mg

For ubiquitin mutants, see page 11; and for ubiquitin chains, see page 15.

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 at least three members, SUMO-1, SUMO-2 and SUMO-3, and SUMO-4, although the role of the latter remains poorly understood. All members are expressed in precursor form and have to 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 three SUMO family members are able to form chains *in vitro*. In cells, SUMOs have the potential to polymerise *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*. However, the functional relevance of SUMO polymerisation is still unclear and much work focuses on the identity of the endogenous target proteins that are conjugated to SUMO polymers. [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 co-ordination of these two systems is a critical contributor to the regulation of many fundamental cellular events.

LITERATURE REFERENCE:
[1] Small ubiquitin-related modifiers in chains: A.C. Vertegaal; Biochem. Soc. Trans. **35,** 1422 (2007)

Product	Utility	Prod. No.	Size
pro-SUMO-1, His_6-tagged	For regulation and processing studies	BML-UW9190-0500	500 µg
SUMO-1, His_6-tagged	Mature protein for functional studies	BML-UW9195-0500	500 µg
SUMO-1, GST-tagged		BML-UW0160-0500	500 µg
SUMO-1, agarose conjugate	For affinity purification of SUMO-1 interacting proteins	BML-UW0095-0500	0.5 ml

Product	Utility	Prod. No.	Size
pro-SUMO-2, His_6-tagged	For regulation and processing studies	BML-UW9200-0500	500 µg
SUMO-2, His_6-tagged	Mature protein for functional studies	BML-UW9205-0500	500 µg
SUMO-2, GST-tagged		BML-UW0165-0500	500 µg
SUMO-2, agarose conjugate	For affinity purification of SUMO-2 interacting proteins	BML-UW0100-0500	0.5 ml
pro-SUMO-3, His_6-tagged	For regulation and processing studies	BML-UW9210-0500	500 µg
SUMO-3, His_6-tagged	Mature protein for functional studies	BML-UW9215-0500	500 µg
SUMO-3, GST-tagged		BML-UW0170-0500	500 µg
SUMO-3, agarose conjugate	For affinity purification of SUMO-3 interacting proteins	BML-UW0105-0500	0.5 ml

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* [1,2], with mechanisms for formation proposed [3].

LITERATURE REFERENCES:

[1] A targeted proteomic analysis of the ubiquitin-like modifier nedd8 and associated proteins: J. Jones, et al.; J. Proteome Res. **7**, 1274 (2008)
[2] Novel substrates and functions for the ubiquitin-like molecule NEDD8: D.P. Xirodimas; Biochem. Soc. Trans. **36,** 802 (2008)
[3] The mechanism of poly-NEDD8 chain formation in vitro: Y. Ohki, et al.; BBRC **381,** 443 (2009)

Product	Utility	Prod. No.	Size
pro-NEDD8, His_6-tagged	For regulation and processing studies	BML-UW9220-0500	500 µg
pro-NEDD8, GST-tagged		BML-UW8740-0100	100 µg
NEDD8, His_6-tagged	Mature protein for functional studies	BML-UW9225-0500	500 µg
NEDD8, agarose conjugate	For affinity purification of NEDD8 interacting proteins	BML-UW0110-0500	0.5 ml

ISG15

A lesser 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 [1], although its role in certain disease states such as malignant transformation has recently been proposed [2].

LITERATURE REFERENCES:
[1] ISG15: the immunological kin of ubiquitin: K.J. Ritchie & D.E. Zhang; Semin. Cell Dev. Biol. **15,** 237 (2004)
[2] Expression, regulation and function of the ISGylation system in prostate cancer: A. Kiessling, et al.; Oncogene **28,** 2606 (2009)

Product	Utility	Prod. No.	Size
pro-ISG15, His_6-tagged	For regulation and processing studies	BML-UW9230-0500	500 µg
ISG15, His_6-tagged	Mature protein for functional studies	BML-UW9235-0500	500 µg
ISG15, agarose conjugate	For affinity purification of ISG15 interacting proteins	BML-UW0115-0500	0.5 ml

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. [1]. 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 [2]. Again, as with ISG15 modification, a role in malignant transformation has been proposed [3].

LITERATURE REFERENCES:
[1] FAT10, a ubiquitin-independent signal for proteasomal degradation: M.S. Hipp, et al.; Mol. Cell Biol. **25,** 3483 (2005).
[2] 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)
[3] 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	Utility	Prod. No.	Size
FAT10, His_6-tagged	For functional studies	BML-UW9240-0250	250 µg
FAT10, agarose conjugate	For affinity purification of FAT10 interacting proteins	BML-UW0140-0500	0.5 ml

Miscellaneous Ubls

Product	Utility	Prod. No.	Size
pro-Ubl5, His_6-tagged	For regulation and processing studies	BML-UW9495-0100	100 µg
Ubl5, His_6-tagged	Mature proteins for functional studies	BML-UW9525-0100	100 µg
Urm1, His_6-tagged		BML-UW9530-0100	100 µg
Fub1 (FUB1), His_6-tagged		BML-UW9535-0100	100 µg

Ubiquitin & Ubl Mutants

Product	Utility	Prod. No.	Size
Ubiquitin^{+1}	Recombinant frame-shift extended protein	BML-UW8790-0100	100 µg
Ubiquitin$_5^{+1}$	Polyubiquitinylated Ub^{+1}	BML-UW8855-0025	25 µg
[D^{77}]Ubiquitin	Incapable of C-terminal isopeptide bond formation	BML-UW0345-0001	1 mg
[K^{0}]Ubiquitin	Negative control for poly-ubiquitinylation experiments	BML-UW0205-1000	1 mg
[K^{6}-only]Ubiquitin	For production of poly-ubiquitin chains *via* Lys^{6} only	BML-UW0210-0001	1 mg
[K^{11}-only]Ubiquitin	For production of poly-ubiquitin chains *via* Lys^{11} only	BML-UW0215-0001	1 mg
[K^{27}-only]Ubiquitin	For production of poly-ubiquitin chains *via* Lys^{27} only	BML-UW0220-0001	1 mg
[K^{29}-only]Ubiquitin	For production of poly-ubiquitin chains *via* Lys^{29} only	BML-UW0225-0001	1 mg
[K^{33}-only]Ubiquitin	For production of poly-ubiquitin chains *via* Lys^{33} only	BML-UW0230-0001	1 mg
[K^{48}-only]Ubiquitin	For production of poly-ubiquitin chains *via* Lys^{48} only	BML-UW0235-0001	1 mg
[K^{63}-only]Ubiquitin	For production of poly-ubiquitin chains *via* Lys^{63} only	BML-UW0240-0001	1 mg
[K^{6}R]Ubiquitin	For production of poly-ubiquitin chains *via* all lysines except Lys^{6}	BML-UW0245-0001	1 mg
[K^{11}R]Ubiquitin	For production of poly-ubiquitin chains *via* all lysines except Lys^{11}	BML-UW0250-0001	1 mg
[K^{27}R]Ubiquitin	For production of poly-ubiquitin chains *via* all lysines except Lys^{27}	BML-UW0255-0001	1 mg
[K^{29}R]Ubiquitin	For production of poly-ubiquitin chains *via* all lysines except Lys^{29}	BML-UW0260-0001	1 mg
[K^{33}R]Ubiquitin	For production of poly-ubiquitin chains *via* all lysines except Lys^{33}	BML-UW0265-0001	1 mg
[K^{48}R]Ubiquitin	For production of poly-ubiquitin chains *via* all lysines except Lys^{48}	BML-UW8615-0001	1 mg
[K^{63}R]Ubiquitin	For production of poly-ubiquitin chains *via* all lysines except Lys^{63}	BML-UW0275-0001	1 mg

[Lys/Arg]Ubiquitin Mutants

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	Utility	Prod. No.	Size
[E^{93}R]SUMO-1, GST-tagged	For use in proteomic studies	BML-UW0175-0100	100 µg
[K^{11}R]SUMO-2, GST-tagged	Incapable of forming SUMO-2 chains at Lys^{11}	BML-UW0380-0100	100 µg
[K^{11}R]SUMO-2		BML-UW0515-0100	100 µg
[K^{11}R]SUMO-3, GST-tagged	Incapable of forming SUMO-3 chains at Lys^{11}	BML-UW0385-0100	100 µg
[K^{11}R]SUMO-3		BML-UW0520-0100	100 µg

Ubiquitin & Ubl N-terminal and Side Chain Derivatives

Product	Utility	Prod. No.	Size
Ubiquitin, [N$^{\varepsilon}$-biotinyl-Lys^{6}]	For detection and purification of ubiquitinylated substrates	BML-UW8470-0100	100 µg
Ubiquitin, [N$^{\varepsilon}$-biotinyl-Lys^{6}, N$^{\varepsilon}$-biotinyl-Lys^{48}]		BML-UW8475-0100	100 µg
Ubiquitin, [N$^{\varepsilon}$-biotinyl-Lys^{6}, N$^{\varepsilon}$-biotinyl-Lys^{63}]		BML-UW8480-0100	100 µg
Ubiquitin, biotinylated (randomly)		BML-UW8705-0100	100 µg
Ubiquitin, methylated	Incapable of forming poly-ubiquitin chains *via* lysine linkages	BML-UW8555-0001	1 mg
SUMO-1, biotinylated (randomly)	For detection and purification of SUMOylated substrates	BML-UW0545-0100	100 µg
SUMO-2, biotinylated (randomly)		BML-UW0550-0100	100 µg
SUMO-3, biotinylated (randomly)		BML-UW0555-0100	100 µg
NEDD8, biotinylated (randomly)	For detection and purification of NEDDylated substrates	BML-UW0560-0100	100 µg

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$^{\alpha}$-amino group as well as on lysine N$^{\varepsilon}$-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.

Technical Note

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$^{\alpha}$- or N$^{\varepsilon}$-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 analysed under stringent conditions in order to ensure the integrity of the material supplied.

Technical Note

Ubiquitin & Ubl C-terminal and Side Chain Derivatives

Product	Utility	Prod. No.	Size
Ubiquitin-AMC	Fluorogenic substrate for deubiquitinylating enzymes (DUBs)	BML-SE211-0025	25 µg
Ubiquitin aldehyde	Potent and specific inhibitor of ubiquitin C-terminal hydrolases (UCHs)	BML-UW8450-0050	50 µg
Ubiquitin vinyl sulphone, HA-tagged (HA-Ub-VS)	Covalent inhibitors for detection and identification of deubiquitinylating enzymes (DUBs)	BML-UW0155-0025	25 µg
Ubiquitin vinyl methyl ester, HA-tagged (HA-Ub-VME)		BML-UW0880-0025	25 µg
Chloroethyl-ubiquitin, HA-tagged (HA-Ub-Cl)		BML-UW0885-0025	25 µg
Bromoethyl-ubiquitin, HA-tagged (HA-Ub-Br)		BML-UW0890-0025	25 µg

HA-Ubiquitin Vinyl Sulphone

For the detection and identification of deubiquitinylating 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.

LITERATURE REFERENCES:

A novel active site-directed probe specific for deubiquitylating enzymes reveals proteasome association of USP14: A. Borodovsky, et al.; EMBO **20,** 5187 (2001)
Chemistry-based functional proteomics reveals novel members of the deubiquitinating enzyme family: A. Borodovsky, et al.; Chem. Biol. **9,** 1149 (2002)
Derivitization of the C-terminus of ubiquitin and ubiquitin-like proteins using intein chemistry: methods and uses: K.D. Wilkinson, et al.; Methods Enzymol. **399,** 37 (2005)

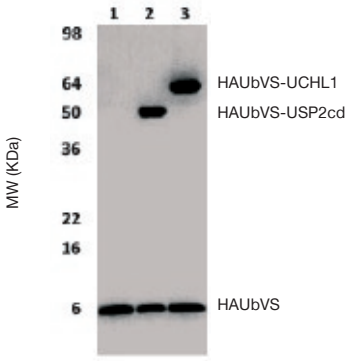

FIGURE: DUB active site probe assay: Western blot showing reactions containing HAUbVS only (lane 1), HAUbVS + USP2cd (lane 2, Prod. No. BML-UW9850), and HAUbVS + GSTUCHL1 (lane 3, Prod. No. BML-UW9305), HAUbVS modified proteins detected using HA-reactive polyclonal antibody (Sigma - H6908) at 1:2000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

Product	Utility	Prod. No.	Size
SUMO-1 aldehyde	Specific inhibitors of deSUMOylating enzymes	BML-UW0060-0025	25 µg
SUMO-2 aldehyde		BML-UW0065-0025	25 µg
SUMO-1-AMC	Fluorogenic substrates for deSUMOylating enzymes	BML-UW0040-0025	25 µg
SUMO-1 [93-97]-AMCA		BML-UW0500-1000	1 mg
SUMO-2-AMC		BML-UW0045-0025	25 µg
NEDD8 aldehyde	Potent, specific and reversible inhibitor of deNEDDylating enzymes	BML-UW0070-0050	50 µg
NEDD8-AMC	Fluorogenic substrate for deNEDDylating enzymes	BML-UW0050-0025	25 µg

Ubiquitin & Ubl Chains

Product	Utility	Prod. No.	Size
Ubiquitin$_5^{+1}$	Polyubiquitinylated Ub^{+1}	BML-UW8855-0025	25 µg
Di-ubiquitin (Ub$_2$), K^{48}-linked	Substrates for deubiquitinylating enzyme assays and polyubiquitin binding studies	BML-UW9800-0100	100 µg
Di-ubiquitin (Ub$_2$), K^{63}-linked		BML-UW0730-0100	50 µg
Tri-ubiquitin (Ub$_3$), K^{63}-linked		BML-UW0745-0100	50 µg
Tetra-ubiquitin (Ub$_4$), K^{48}-linked		BML-UW8645-0025	25 µg
Tetra-ubiquitin (Ub$_4$), K^{63}-linked		BML-UW0715-0025	25 µg
Poly-ubiquitin chains (Ub$_{2-7}$), K^{48}-linked		BML-UW8860-0100	100 µg
Poly-ubiquitin chains (Ub$_{2-7}$), K^{63}-linked		BML-UW9570-0100	100 µg
Poly-ubiquitin chains (Ub$_{2-16}$), K^{48}-linked		BML-UW0670-0100	100 µg
([K^{wt}]Ub)$_n$-ubiquitinylated substrate		BML-UW0610-0025	25 µg
([K^6 only]Ub)$_n$-ubiquitinylated substrate		BML-UW0615-0025	25 µg
([K^{11} only]Ub)$_n$-ubiquitinylated substrate		BML-UW0620-0025	25 µg
([K^{27} only]Ub)$_n$-ubiquitinylated substrate		BML-UW0625-0025	25 µg
([K^{29} only]Ub)$_n$-ubiquitinylated substrate		BML-UW0630-0025	25 µg
([K^{33} only]Ub)$_n$-ubiquitinylated substrate		BML-UW0635-0025	25 µg
([K^{48} only]Ub)$_n$-ubiquitinylated substrate		BML-UW0640-0025	25 µg
([K^{63} only]Ub)$_n$-ubiquitinylated substrate		BML-UW0645-0025	25 µg

Ubiquitin Chains

Useful as standards for chain synthesis, recognition, breakdown studies, for deubiquitinylating enzyme assays and polyubiquitin binding studies. Amongst other applications, the novel single isopeptide linkage-based polyubiquitinylated substrate products may find great utility for the detailed study of deconjugating enzyme and ubiquitin binding domain specificities. They have already proven of considerable utility in assisting in the definition of the isopeptide-linkage specificity of an ubiquitin-reactive monoclonal antibody [1].

LITERATURE REFERENCE:

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

Ubiquitin & Ubl Reactive Antibodies

Whilst there are a great number of antibodies available that are capable of recognising ubiquitin or other members of the Ubl family, there are few that are as well described in the scientific literature as the monoclonal antibodies BML-PW8805 and BML-PW8810 [clones FK1 and FK2]. These antibodies are capable of recognising mono- and/or polyubiquitinylated species and, when used in concert, are capable of discriminating these modification types. The introduction of the K^{63}-linkage specific monoclonal antibody BML-PW0600 [clone HWA4C4] [1] signalled the very first commercially available ubiquitin isopeptide-linkage specific reagent. Such immunological tools are of huge value in the determination of ubiquitinylation status in a variety of applications.

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

Product	Specificity	Application	Prod. No.	Size
Ubiquitin-protein conjugates, pAb	Species independent	IHC, WB	BML-UG9510-0025 BML-UG9510-0100	25 µl 100 µl
Polyubiquitinylated conjugates, mAb [clone FK1]	Species independent	IHC, WB	BML-PW8805-0500	500 µg
Mono- and polyubiquitinylated conjugates, mAb [clone FK2]	Species independent	IHC, IP, WB	BML-PW8810-0500	500 µg
Mono- and polyubiquitinylated conjugates, mAb [clone FK2] HRP conjugate	Wide range of species	ELISA, IHC, WB	BML-PW0150-0025 BML-PW0150-0100	25 µg 100 µg
Polyubiquitin (K^{63}-linkage-specific), mAb [clone HWA4C4]	Wide range of species	ELISA, IHC, ICC, WB	BML-PW0600-0025 BML-PW0600-0100	25 µl 100 µl
Polyubiquitin (K^{63}-linkage-specific), mAb [clone HWA4C4] HRP conjugate	Wide range of species	WB	BML-PW0605-0025 BML-PW0605-0100	25 µl 100 µl
Ub^{+1}, pAb	Human	WB	BML-PW9780-0025 BML-PW9780-0100	25 µl 100 µl

Ubiquitin-reactive Antibodies

BML-PW8805-0500 500 µg
BML-PW8810-0500 500 µg

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 polyubiquitinylated proteins and not monoubiquitinylated proteins or free ubiquitin, whilst clone FK2 recognizes both mono- and poly-ubiquitinylated species but not free ubiquitin. By using these antibodies in concert the degree of protein ubiq-uitinylation may be determined.

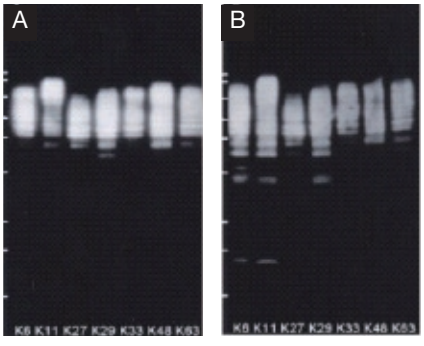

FIGURE: Immunodetection of single lysine linked polyubiquitin chains by western bloting following SDS-PAGE using [A] BML-PW8805 (clone FK1) and [B] BML-PW8810 (clone FK2).

K^{63}-linkage-specific Ubiquitin-reactive Antibody

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

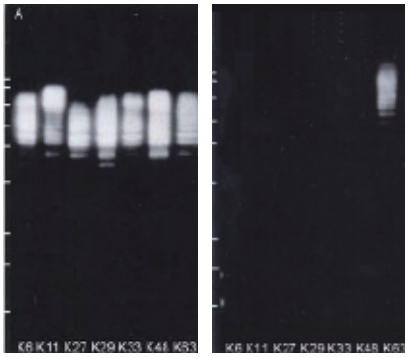

FIGURE: Western blot following SDS-PAGE of single lysine mutant chains probed with pan-reactive mAb FK1 (BML-PW8805) & K^{63}-linkage specific mAb HWA4C4 (BML-PW0600).

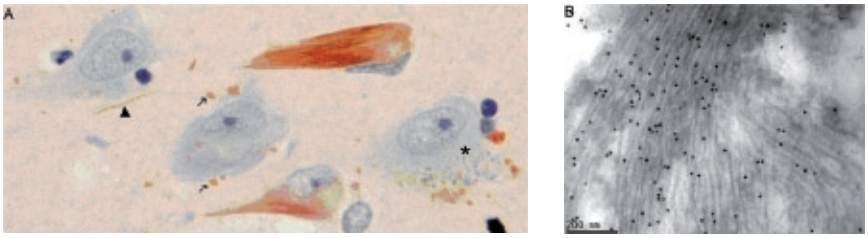

FIGURE: [A] Sections through the hippocampus in Alzheimer's Disease (AD), stained using mAb HWA4C4 (BML-PW0600) and showing differential staining of neurofibrillary tangles (NFTs). [B] Immunogold labelling TEM in AD using HWA4C4 mAb (BML-PW0600) provides evidence that K^{63}-linked polyubiquitin is present in NFTs [1].

LITERATURE REFERENCE:

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

Product	Specificity	Application	Prod. No.	Size
SUMO-1, pAb	Human, mouse, rat	IHC, IP, WB	ALX-210-174-R200	200 µl
SUMO-1, sheep pAb	Human	ICC, IP, WB	BML-PW0505-0025 BML-PW0505-0100	25 µl 100 µl
SUMO-1 (N-terminus), pAb	Human	WB	BML-PW8330-0025 BML-PW8330-0100	25 µl 100 µl
SUMO-1 (C-terminus), pAb	Human	WB	BML-PW9460-0025 BML-PW9460-0100	25 µl 100 µl
SUMO-2/-3, sheep pAb	Human	ICC, IP, WB	BML-PW0510-0025 BML-PW0510-0100	25 µl 100 µl
SUMO-2/-3 (N-terminus), pAb	Human	WB	BML-PW9465-0025 BML-PW9465-0100	25 µl 100 µl
NEDD8, pAb	Human	IP, WB	BML-PW9340-0025 BML-PW9340-0100	25 µl 100 µl
NEDD8, pAb	Human, mouse, rat	IHC, ICC, IP, WB	ALX-210-194-R200	200 µl
ISG15, pAb	Human	WB	BML-PW9575-0025 BML-PW9575-0100	25 µl 100 µl
FAT10, pAb (protein-derived)	Human, mouse	IP, WB	BML-PW9680-0025 BML-PW9680-0100	25 µl 100 µl
FAT10, pAb (peptide-derived)	Human, mouse	IP, WB	BML-PW9585-0025 BML-PW9585-0100	25 µl 100 µl
Ubl5, pAb	Human	WB	BML-PW9605-0025 BML-PW9605-0100	25 µl 100 µl
Urm1, pAb	Human	IHC, WB	BML-PW9595-0025 BML-PW9595-0100	25 µl 100 µl
Fub1, pAb	Human	WB	BML-PW9615-0025 BML-PW9615-0100	25 µl 100 µl

Ubiquitin & Ubl Cascade Enzymes

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 approaching 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 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, is usually facilitated by a ligase enzyme (E3) [see Figure 5 on Page 6]. 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.

Activating Enzymes (E1s) – Proteins

Product	Utility	Prod. No.	Size
Ubiquitin activating enzyme UBE1L, His_6-tagged	Ubiquitin-specific activation	BML-UW9410-0050	50 µg
Ubiquitin activating enzyme UBE1L (rabbit)		ALX-202-048-C025	25 µg
SUMO activating enzyme, His_6-tagged	SUMO-specific activation	BML-UW9330-0025	25 µg
NEDD8 activating enzyme, His_6-tagged	NEDD8-specific activation	BML-UW9950-0025	25 µg
ISG15 activating enzyme, His_8-tagged	ISG15-specific activation	BML-UW9955-0025	25 µg

Activating Enzymes (E1s) – Antibodies

Product	Specificity	Application	Prod. No.	Size
Ubiquitin activating enzyme E1A, (N-terminus), pAb	Human, rat, mouse, rabbit, chicken, cow	IHC, IP, WB	BML-PW8385-0025 BML-PW8385-0100	25 µl 100 µl
Ubiquitin activating enzyme E1A/B, (N-terminus), pAb	Human, rabbit, chicken, cow	IHC, IP, WB	BML-PW8390-0025 BML-PW8390-0100	25 µl 100 µl
Ubiquitin activating enzyme E1A/B, (C-terminus), pAb	Wide range of species	WB	BML-PW8395-0025 BML-PW8395-0100	25 µl 100 µl
Ubiquitin activating enzyme Ube1L, pAb	Human	IHC (FS, PS), WB	ALX-210-391-R100	100 µl
Ubiquitin activating enzyme Ube1-L2 (UBA6), pAb	Human	WB	BML-PW0525-0025 BML-PW0525-0100	25 µl 100 µl

Conjugating Enzymes (E2s) – Proteins

Product	Prod. No.	Size
Ubiquitin		
UbcH1	BML-UW9735-0100	100 µg
UbcH1, His_6-tagged	BML-UW9020-0100	100 µg
UbcH1, GST-tagged	BML-UW9730-0100	100 µg
UbcH2, His_6-tagged	BML-UW9025-0100	100 µg
UbcH3, His_6-tagged	BML-UW8730-0100	100 µg
UbcH5a, His_6-tagged	BML-UW9050-0100	100 µg
[$C^{85}A$]UbcH5a, His_6-tagged	BML-UW9055-0100	100 µg
UbcH5b	BML-UW0565-0100	100 µg
UbcH5b, His_6-tagged	BML-UW9060-0100	100 µg
[$C^{85}A$]UbcH5b, His_6-tagged	BML-UW9065-0100	100 µg
UbcH5c, His_6-tagged	BML-UW9070-0100	100 µg
[$C^{85}A$]UbcH5c, His_6-tagged	BML-UW9075-0100	100 µg
UbcH6, His_6-tagged	BML-UW8710-0100	100 µg
UbcH7, His_6-tagged	BML-UW9080-0100	100 µg
UbcH8, His_6-tagged	BML-UW9135-0100	100 µg
UbcH10, His_6-tagged	BML-UW8715-0100	100 µg
UbcH12, His_6-tagged	BML-UW9145-0100	100 µg
UbcH13/Mms2, His_6-tagged	BML-UW9565-0100	100 µg
hHR6A, His_6-tagged	BML-UW9635-0100	100 µg
hHR6B, His_6-tagged	BML-UW9640-0100	100 µg
Ubiquitin-conjugating enzyme sampler pack	BML-UW8975-0001	1 Pack
SUMO		
UbcH9	BML-UW9320-0100	100 µg
NEDD8		
UbcH12, His_6-tagged	BML-UW9145-0100	100 µg
ISG15		
UbcH8, His_6-tagged	BML-UW9135-0100	100 µg

Conjugating Enzymes (E2s) – Antibodies

Product	Specificity	Application	Prod. No.	Size
Ubiquitin conjugating enzyme UbcH1, pAb	Human, cow	WB	BML-UG9520-0025 BML-UG9520-0100	25 µl 100 µl
UbcH9, pAb	Species independent	ICC, WB	ALX-210-233-C050	50 µg

Ligases (E3s) – Proteins

Product	Prod. No.	Size
Ubiquitin		
CHIP	ALX-201-215-C025	25 µg
Hdm2 catalytic RING domain, GST-tagged	BML-UW0200-0025	25 µg
Rbx1, His_6-tagged	BML-UW0395-0025	25 µg
MuRF1, GST-tagged (rat, recombinant)	BML-UW0405-0025	25 µg
SUMO		
RanBP2ΔFG fragment, GST-tagged	BML-UW9455-0100	100 µg
PIAS1, GST-tagged	BML-UW9960-0025	25 µg

Ligases (E3s) – Antibodies

Product	Specificity	Application	Prod. No.	Size
DDA1, pAb	Human, mouse	IP, WB	BML-PW0455-0025 BML-PW0455-0100	25 µl 100 µl
DDB1, pAb	Human, mouse	WB	BML-PW0460-0025 BML-PW0460-0100	25 µl 100 µl
AtRbx1, pAb	*Arabidopsis*	WB	BML-PW0465-0025 BML-PW0465-0100	25 µl 100 µl
AtCul3, pAb	*Arabidopsis*	IP, WB	BML-PW0470-0025 BML-PW0470-0100	25 µl 100 µl
COP1, pAb	Human, mouse, rat, hamster, monkey	IP, WB	BML-PW9725-0025 BML-PW9725-0100	25 µl 100 µl
CHIP, pAb	Human	WB	ALX-210-883-C100	100 µg

Deconjugating Enzymes (DCEs) – Proteins

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

Mammals contain some 80-90 deubiquitinylating enzymes (DUBs) falling into five subfamilies, namely the ubiquitin C-terminal hydrolases (UCHs); the ubiquitin-specific peptidases (USPs); the ovarian tumor (OTU) domain proteins; the Josephin or Machado-Joseph disease (MJD) proteins, and the JAMM (Jab1/MPN domain-associated metalloisopeptidase) domain proteases. Most DUBs contain a catalytic domain that has sequence similarity within subfamilies and structural similarity across subfamilies, and unrelated sequences either N-terminal or C-terminal (or both) to the catalytic domain. These flanking sequences have been shown to mediate substrate binding and presumably serve as substrate binding domains in all DUBs. They, along with the catalytic core, could also contribute binding and cleavage specificity for different ubiquitin-ubiquitin isopeptide linkages [1].

Since most DUBs have been identified only by means of sequence similarity to catalytic motifs, there is little known functional information on many of these enzymes with only a handful of these DUBs having been characterized with respect to the proteins with which they interact and deubiquitinylate. However, it is becoming increasingly apparent that DUBs must acquire their substrates by binding the target protein in a conjugate or by associating with other macromolecular complexes. Further study may reveal a variety of protein partners including substrates, scaffolds, adaptors and ubiquitin receptors. Much of the regulation and specificity of deubiquitinylation arises from the association of DUBs with these protein partners [2].

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

LITERATURE REFERENCES:

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

Product	Utility	Prod. No.	Size
UCH-L1 (PGP9.5), GST-tagged	Ubiquitin C-terminal hydrolases	BML-UW9305-0050	50 µg
UCH-L1 (PGP9.5), His_6-tagged		BML-UW9740-0050	50 µg
UCH-L3, His_6-tagged		BML-UW9745-0050	50 µg
BAP1, His_6-tagged	BRCA1-associated ubiquitin C-terminal hydrolase	BML-UW9855-0050	50 µg
USP2 catalytic domain, (rat, recombinant)	Ubiquitin specific proteases	BML-UW9850-0100	100 µg
USP5 (isopeptidase T), long form		BML-UW9690-0025	25 µg
USP5 (isopeptidase T), short form		BML-UW9695-0025	25 µg
USP14		BML-UW9840-0100	100 µg
USP15, His_6-tagged		BML-UW9845-0100	100 µg
USP25, isoform 2, His_6-tagged		BML-UW0475-0050	50 µg
Otubain-1, His_6-tagged		BML-UW0680-0100	100 µg
SENP1 catalytic fragment, GST-tagged	SUMO specific proteases	BML-UW9760-0100	100 µg
SENP2 catalytic fragment, GST-tagged		BML-UW9765-0100	100 µg
NEDP1, His_6-tagged	NEDD8-specific protease	BML-UW9770-0100	100 µg
COP9 Signalosome complex	Shows kinase, deubiquitinylating, and de-NEDDylating activities	BML-PW9425-0010	10 µg

Deconjugating Enzymes (DCEs) – Substrates & Inhibitors

Product	Utility	Prod. No.	Size
Ubiquitin			
Z-Leu-Arg-Gly-Gly-AMC	Fluorogenic substrates for deubiquitinylating enzymes (DUBs)	BML-P801-0005	5 mg
Z-Arg-Leu-Arg-Gly-Gly-AMC		BML-ZW8585-0005	5 mg
Ubiquitin-AMC		BML-SE211-0025	25 µg
Ubiquitin$_5^{+1}$	Polyubiquitinylated Ub^{+1}	BML-UW8855-0025	25 µg
Di-ubiquitin (Ub$_2$), K^{48}-linked	Substrates for DUB assays and polyubiquitin binding studies	BML-UW9800-0100	100 µg
Di-ubiquitin (Ub$_2$), K^{63}-linked		BML-UW0730-0050	50 µg
Tri-ubiquitin (Ub$_3$), K^{63}-linked		BML-UW0745-0050	50 µg
Tetra-ubiquitin (Ub$_4$), K^{48}-linked		BML-UW8645-0025	25 µg
Tetra-ubiquitin (Ub$_4$), K^{63}-linked		BML-UW0715-0025	25 µg
Poly-ubiquitin chains (Ub$_{2\text{-}7}$), K^{48}-linked		BML-UW8860-0100	100 µg
Poly-ubiquitin chains (Ub$_{2\text{-}7}$), K^{63}-linked		BML-UW9570-0100	100 µg
Poly-ubiquitin chains (Ub$_{2\text{-}16}$), K^{48}-linked		BML-UW0670-0100	100 µg
([K^{wt}]Ub)$_n$-ubiquitinylated substrate		BML-UW0610-0025	25 µg
([K^6 only]Ub)$_n$-ubiquitinylated substrate		BML-UW0615-0025	25 µg
([K^{11} only]Ub)$_n$-ubiquitinylated substrate		BML-UW0620-0025	25 µg
([K^{27} only]Ub)$_n$-ubiquitinylated substrate		BML-UW0625-0025	25 µg
([K^{29} only]Ub)$_n$-ubiquitinylated substrate		BML-UW0630-0025	25 µg
([K^{33} only]Ub)$_n$-ubiquitinylated substrate		BML-UW0635-0025	25 µg
([K^{48} only]Ub)$_n$-ubiquitinylated substrate		BML-UW0640-0025	25 µg
([K^{63} only]Ub)$_n$-ubiquitinylated substrate		BML-UW0645-0025	25 µg
Ubiquitin aldehyde	Inhibitor of DUBs	BML-UW8450-0050	50 µg
Ubiquitin vinyl sulphone, HA-tagged (HA-Ub-VS)	Covalent inhibitors for detection and identification of deubiquitinylating enzymes (DUBs)	BML-UW0155-0025	25 µg
Ubiquitin vinyl methyl ester, HA-tagged (HA-Ub-VME)		BML-UW0880-0025	25 µg
Chloroethyl-ubiquitin, HA-tagged (HA-Ub-Cl)		BML-UW0885-0025	25 µg
Bromoethyl-ubiquitin, HA-tagged (HA-Ub-Br)		BML-UW0890-0025	25 µg
SUMO			
SUMO-1-AMC	Fluorogenic substrates for de-SUMOylating enzymes	BML-UW0040-0025	25 µg
SUMO-1 [93-97]-AMCA		BML-UW0500-1000	1 mg
SUMO-2-AMC		BML-UW0045-0025	25 µg
SUMO-1 aldehyde	Inhibitors of deSUMOylating enzymes	BML-UW0060-0025	25 µg
SUMO-2 aldehyde		BML-UW0065-0025	25 µg

NEDD8			
NEDD8-AMC	Fluorogenic substrate for deNEDDylating enzymes	BML-UW0050-0025	25 µg
NEDD8 aldehyde	Inhibitor of deNEDDylating enzymes	BML-UW0070-0050	50 µg

Deconjugating Enzymes (DCEs) – Antibodies

Product	Specificity	Application	Prod. No.	Size
UCH-L1 (PGP9.5), pAb	Human, mouse, rat	IHC, WB	BML-PG9500-0025 BML-PG9500-0100	25 µl 100 µl
USP7 (HAUSP), pAb	Human	WB	BML-PW0540-0025 BML-PW0540-0100	25 µl 100 µl
USP15, pAb	Human, mouse	WB	BML-PW9795-0025 BML-PW9795-0100	25 µl 100 µl
USP21, pAb	Human	WB	BML-PW0585-0025 BML-PW0585-0100	25 µl 100 µl
AMSH (STAMBP), pAb	Human	WB	BML-PW0655-0025 BML-PW0655-0100	25 µl 100 µl
MYSM1, pAb	Human	WB	BML-PW0660-0025 BML-PW0660-0100	25 µl 100 µl
SENP1, pAb	Human, mouse	WB	ALX-210-862-R200	200 µl
SENP2, pAb	Human, mouse	WB	ALX-210-863-R200	200 µl
SENP2, pAb	Mouse	ELISA, WB	ALX-210-482-C100	100 µg
SENP5, pAb	Human	WB	BML-PW0365-0025 BML-PW0365-0100	25 µl 100 µl
SENP6, sheep pAb	Human	WB	BML-PW0370-0025 BML-PW0370-0100	25 µl 100 µl
NEDP1, pAb	Human	WB	BML-PW9775-0025 BML-PW9775-0100	25 µl 100 µl
CYLD, pAb	Human	ICC, WB	ALX-210-910-C050	50 µg
CYLD, pAb	Human	ICC, WB	BML-PW0760-0025 BML-PW0760-0100	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 [1-3]. PGP9.5 is a member of the ubiquitin C-terminal hydrolase family [4,5] and immunohistochemical studies have shown that the protein is enriched in several ubiquinated inclusion bodies, suggesting that such structures may be metabolically dynamic regions of the cell [6]. The antiserum may be used in Western blotting (24kDa) [7] and has been used on para-formaldehyde-fixed cryostat, Vibratome and dewaxed tissue sections, at dilutions up to 1:4000 when used in combination with sensitive detection methods, such as ABC-peroxidase (Vector-Elite).

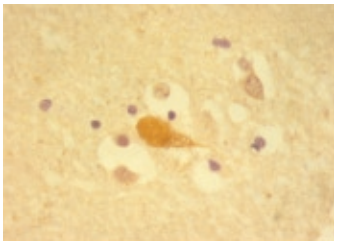

FIGURE: Cortical Lewy body in human brain immunostained using the rabbit antiserum to PGP9.5 (BML-PG9500). Micrograph courtesy of Prof. RJ Mayer (University of Nottingham).

LITERATURE REFERENCES:

[1] PGP 9.5--a new marker for vertebrate neurons and neuroendocrine cells: R.J. Thompson, et al.; Brain Res. **278,** 224 (1983)
[2] 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)
[3] Protein gene product (PGP) 9.5 in diagnostic (neuro-) oncology. An immunomorphological study: B. Ermisch & K. Schwechheimer; Clin. Neuropathol. **14,** 130 (1995)
[4] The neuron-specific protein PGP 9.5 is a ubiquitin carboxyl-terminal hydrolase: K.D. Wilkinson, et al.; Science **246,** 670 (1989)
[5] 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)
[6] 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)
[7] 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

NF-κB and IKKα

The regulation of all events in the NF-κB 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-κB 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-κB factors are retained in an inactive state by binding to the inhibitor of NF-κB (IκB) which, in response to cell stimulation, is ubiquitinylated (by derivatisation with K^{48}-linked chains) and degraded by the proteasome. Prior to its ubiquitinylation, IκB is phosphorylated by the IκB kinase (IKK) complex. The IKK complex, consisting of two kinases, IKKα and IKKβ, and the regulatory component NEMO, is activated by an upstream kinase (TAK1) which is in turn activated after TNFα or IL-1 receptor stimulation.

LITERATURE REFERENCES:

[1] Ubiquitin signals in the NF-kappaB pathway: J. Terzic, et al.; Biochem. Soc. Trans. **35,** 942 (2007)
[2] Linear polyubiquitination: a new regulator of NF-kappaB activation: K. Iwai & F. Tokunaga; EMBO Rep. **10,** 706 (2009)
[3] The role of ubiquitin in NF-kappaB regulatory pathways: B. Skaug, et al.; Annu. Rev. Biochem. **78,** 769 (2009)

Product	Detail/Use	Prod. No.	Size
IκBα, GST-tagged	Ubiquitinylation/SUMOylation substrates	BML-UW9970-0050	50 µg
IκBα		BML-UW9975-0050	50 µg
NF-κB p50, His_6-tagged		ALX-201-285-C002	2 µg
NF-κB p50, GST-tagged		BML-UW9980-0050	50 µg
NF-κB p50		BML-UW9985-0050	50 µg
NF-κB p65, His_6-tagged		ALX-201-284-C002	2 µg
NF-κB p65, GST-tagged		BML-UW9990-0050	50 µg
NF-κB p65		BML-UW9995-0050	50 µg

p53

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. In contrast, SUMO and NEDD8 modifications have been shown, respectively, to activate and inhibit p53 transcriptional activity.

LITERATURE REFERENCE:

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

p53 – Proteins

Product	Detail/Use	Prod. No.	Size
p53, His_6-tagged	Ubiquitinylation/SUMOylation substrates	BML-FW8820-0020	20 µg
p53, GST-tagged		BML-FW9370-0050	50 µg
Hdm2 catalytic RING domain, GST-tagged	Ubiquitinylation substrate	BML-UW0200-0025	25 µg

p53 – Antibodies

Product	Specificity	Application	Prod. No.	Size
p53, mAb [clone DO-1]	Human	ELISA, FC, IHC, IP, WB	BML-PW1090-0025	25 µg
p53, mAb [clone DO-7]	Human	ELISA, FC, IHC, IP, WB	BML-PW1095-0025	25 µg
p53, mAb [clone EX-1]	Human	WB	BML-PW1105-0025	25 µg
p53, mAb [clone EX-2]	Human	WB	BML-PW1100-0025	25 µg
p53, mAb [clone EX-3]	Human	WB	BML-PW1115-0025	25 µg
p53, mAb [clone EX-4]	Human	WB	BML-PW1110-0025	25 µg
p53, mAb [clone 1801]	Human	ELISA, FC, IHC, IP, WB	BML-PW1085-0025	25 µg
p53, mAb [clone 421]	Human, mouse, rat	IHC, ICC, IP, WB	BML-SA293-0050	50 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 obscure. 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 an obvious 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 [1]. The following proteins may act as substrates for SUMO modification in combination with the necessary activating and conjugation enzymes.

LITERATURE REFERENCE:

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

SUMOylation Substrates – Proteins

Product	Prod. No.	Size
IRF2, His_6-tagged	BML-UW0335-0100	100 µg
PML SUMOylation motif, GST-tagged	BML-UW9965-0100	100 µg
RanGAP1 fragment [418-587], GST-tagged	BML-UW9755-0100	100 µg
SP100 fragment [241-360], GST-tagged	BML-UW9825-0100	100 µg

SUMOylation Substrates – Antibodies

Product	Specificity	Application	Prod. No.	Size
RanGAP1, pAb	Human	WB	BML-PW8785-0025 BML-PW8785-0100	25 µl 100 µl
SP100, pAb	Human	ICC, WB	BML-PW0325-0025 BML-PW0325-0100	25 µl 100 µl
SUMO-SP100, pAb	Human	ICC	BML-PW0330-0025 BML-PW0330-0100	25 µl 100 µl

Detection & Isolation Kits & Components

In pursuing the development of key reagents for the detection, isolation, purification, and characterisation 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 & BML-UW8955), UbiQapture™-Q Kit (Prod. No. BML-UW8995), for the isolation of mono- and polyubiquitinylated species, various agarose-immobilised ubiquitin binding domains for investigation of ubiquitin binding parameters, and a comprehensive range of Ubl-specific antibodies facilitating study in a variety of applications. The product range is now extended further 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 utilised in conjugation procedures to produce an agarose immobilized complex.

Ubiquitin & Ubl Agarose Conjugates

Product	Detail/Use	Prod. No.	Size
Ubiquitin, agarose conjugate	For protein interaction studies	BML-UW8630-0500	0.5 ml
SUMO-1, agarose conjugate		BML-UW0095-0500	0.5 ml
SUMO-2, agarose conjugate		BML-UW0100-0500	0.5 ml
SUMO-3, agarose conjugate		BML-UW0105-0500	0.5 ml
NEDD8, agarose conjugate		BML-UW0110-0500	0.5 ml
ISG15, agarose conjugate		BML-UW0115-0500	0.5 ml
FAT10, agarose conjugate		BML-UW0140-0500	0.5 ml

Ubiquitin Binding Domains

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

The >16 thus far characterized UBDs are in general 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. Nonhelical 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. For review see [1].

LITERATURE REFERENCE:

[1] Functional roles of ubiquitin-like domain (ULD) and ubiquitin-binding domain (UBD) containing proteins. C. Grabbe & I. Dikic; Chem. Rev., **109,** 1481-94 (2009)

Proteins

Product	Detail/Use	Prod. No.	Size
19S Subunit S5a (Rpn 10), GST-tagged	For binding studies of interacting proteins	BML-UW8465-0100	100 µg
19S Subunit S5a (Rpn 10), agarose conjugate		BML-UW8635-0500	0.5 ml
S5a UIM, agarose conjugate		BML-UW9820-0500	0.5 ml
p62 UBA domain, agarose conjugate		BML-UW9010-0500	0.5 ml
UQ1 UBA domain, agarose conjugate		BML-UW9830-0500	0.5 ml
hHR23B UBA2 domain, agarose conjugate		BML-UW9440-0500	0.5 ml
Dsk2 UBA domain, agarose conjugate		BML-UW9835-0500	0.5 ml
NUB1/NUB1L UBA domain, agarose conjugate		BML-UW9700-0500	0.5 ml
NBR1 UBA domain, agarose conjugate		BML-UW9445-0500	0.5 ml
VPS9 CUE domain, agarose conjugate		BML-UW9450-0500	0.5 ml
Ubiquitin binding entities sampler pack		BML-UW0120-0001	1 Pack

Antibodies

Product	Specificity	Application	Prod. No.	Size
p62 (SQSTM1), pAb	Human	IHC, WB	BML-PW9860-0025 BML-PW9860-0100	25 µl 100 µl
NUB1/1L, pAb	Human	WB	BML-PW9685-0025 BML-PW9685-0100	25 µl 100 µl

Detection and Isolation Kits

Whilst 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. An 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	Detail/Use	Prod. No.	Size
Ubiquitin activating kit		BML-UW0400-0001	1 Kit
HeLa-derived ubiquitin conjugation/ degradation kit	For generating ubiquitinylated proteins	BML-UW9915-0001	1 Kit
Ubiquitinylation kit	For generating ubiquitin-E2 thioesters	BML-UW9920-0001	1 Kit
SUMOylation kit	For generating SUMOylated proteins	BML-UW8955-0001	1 Kit
NEDDylation kit	For generating NEDD8-E2 thioesters	BML-UW0590-0001	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.

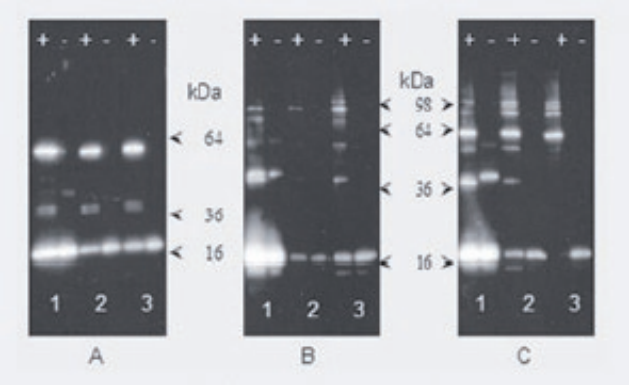

FIGURE: Western blots following SDS-PAGE of SUMOylation assays using: A: RANGAP1 (BML-UW9755); B: SP100 (BML-UW9825); and C: p53 (BML-FW9370) as substrate proteins with the three SUMOs assayed in the presence (+) and absence (-) of ATP– lane 1: SUMO1 (BML-UW9195); lane 2: SUMO2 (BML-UW9205); and lane 3: SUMO3 (BML-UW9215). Detection was with the appropriate SUMO antibodies (SUMO-1: BML-PW9460, SUMO-2/3: BML-PW9465).

Product	Detail/Use	Prod. No.	Size
UbiQapture™-Q kit	For isolation and enrichment of ubiquitinylated proteins	BML-UW8995-0001	1 Kit

UbiQapture Kit

A kit specifically developed for the isolation and enrichment of ubiquitinylated proteins. The kit facilitates the isolation of both mono- and poly-ubiquitinylated proteins (independent of lysine residue chain linkage) from cell extracts, tissue lysates and *in vitro* assay solutions through the use of a broad spectrum affinity matrix. Captured proteins may be analyzed by Western blotting using the highly sensitive ubiquitin-conjugate specific antibody provided, using antibodies to specific proteins of interest, or eluted from the matrix for subsequent biochemical characterization. The UbiQapture™-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 25 binding assays.

Suggested uses

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

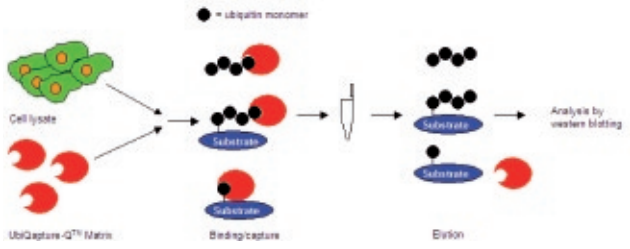

FIGURE: Schematic overview of UbiQapture Kit isolation and detection process.

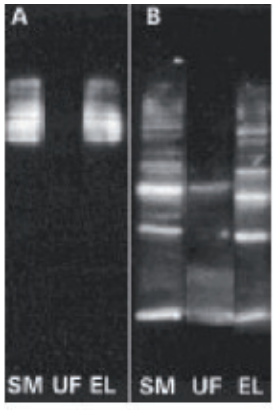

FIGURE: Western blot analysis demonstrating ubiquitin enrichment of partially purified and lysate-derived ubiquitinylated proteins after UbiQapture. Ubiquitin-protein conjugates present in starting material, unbound fraction and elution fraction were detected by western blotting using the provided ubiquitin-conjugate specific HRP-linked antibody (BML-KW0150) at a dilution of 1:1000 dilution. A: Capture of ubiquitinylated UbcH5a from *in vitro* ubiquitinylation assay. B: Capture of Ub-protein conjugates from control ubiquitinylated-protein lysate (BML-UW0130). Key: SM = Starting Material, UF = Unbound Fraction and EL = Elution Fraction.

Kit Components and Reagents

Product	Detail/Use	Prod. No.	Size
Fraction I (FrI, HeLa)	For ubiquitinylation assays and *in vitro* conjugation experiments	BML-HW8600-0001	1 mg
Fraction II (FrII, HeLa)		BML-HW8605-0001	1 mg
HeLa S100 fraction	For demonstrating ubiquitin-proteasome mediated conjugation/degradation	BML-SW8750-0001	1 mg
10 x Ubiquitinylation kit buffer	Assay buffer from the Ubiquitinylation Kit	BML-KW9885-0005	5 ml
10 x SUMOylation kit buffer	Assay buffer from the SUMOylation Kit	BML-KW9890-0005	5 ml
Mg^{2+}/ATP activating solution	To facilitate efficient conjugation and degradation studies	BML-EW9805-0100	100 µl
ATP (energy) regeneration solution		BML-EW9810-0100	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	Prod. No.	Size
11S Activator complex	BML-PW9420-0025	25 µg
11S Subunit α, GST-tagged	BML-PW9120-0100	100 µg
11S Subunit α	BML-PW9865-0100	100 µg
11S Subunit β, GST-tagged	BML-PW9125-0100	100 µg
11S Subunit β	BML-PW9870-0100	100 µg
11S Subunit γ, GST-tagged	BML-PW9130-0100	100 µg
11S Subunit γ	BML-PW9875-0100	100 µg

11S Activator – Antibodies

Product	Specificity	Application	Prod. No.	Size
11S Subunit α, pAb	Human, mouse, rat	IHC, WB	BML-PW8185-0025 BML-PW8185-0100	25 µl 100 µl
11S Subunit β, pAb	Human, mouse, rat	IHC, WB	BML-PW8240-0025 BML-PW8240-0100	25 µl 100 µl
11S Subunit γ, pAb	Human, mouse	IHC, WB	BML-PW8190-0025 BML-PW8190-0100	25 µl 100 µl
11S Subunit antibodies, sampler pack			BML-PW8915-0001	3 x 10 µl

Miscellaneous Activator Complexes - Antibodies

Product	Specificity	Application	Prod. No.	Size
Blm10, pAb	*Saccharomyces cervisiae*	WB	BML-PW0570-0025 BML-PW0570-0100	25 µl 100 µl
POMP, pAb	Human, rat, monkey	IHC, IP, WB	BML-PW9715-0025 BML-PW9715-0100	25 µl 100 µl

19S Regulator ATPase Subunits – Antibodies

Product	Specificity	Application	Prod. No.	Size
19S Subunit Rpt1 (S7), pAb	Yeast	WB	BML-PW8255-0025 BML-PW8255-0100	25 µl 100 µl
19S Subunit Rpt1 (S7), pAb	Human, yeast	WB	BML-PW8165-0025 BML-PW8165-0100	25 µl 100 µl
19S Subunit Rpt1 (S7), pAb	Human	WB	BML-PW8315-0025 BML-PW8315-0100	25 µl 100 µl
19S Subunit Rpt1 (S7), mAb [clone MSS1-104]	Human, mouse	WB	BML-PW8825-0025 BML-PW8825-0100	25 µl 100 µl
19S Subunit Rpt1 (S7), mAb [clone MSS1-92]	Human	IP	BML-PW9400-0025 BML-PW9400-0100	25 µl 100 µl
19S Subunit Rpt2 (S4), pAb	Wide range of species	IHC, WB	BML-PW8160-0025 BML-PW8160-0100	25 µl 100 µl
19S Subunit Rpt2 (S4), pAb	Yeast	WB	BML-PW8260-0025 BML-PW8260-0100	25 µl 100 µl
19S Subunit Rpt2 (S4), pAb	Human, mouse	WB	BML-PW8305-0025 BML-PW8305-0100	25 µl 100 µl
19S Subunit Rpt2 (S4), pAb	*Arabidopsis*	WB	BML-PW0445-0025 BML-PW0445-0100	25 µl 100 µl
19S Subunit Rpt3 (S6b), pAb	Human, yeast	IHC, WB	BML-PW8250-0025 BML-PW8250-0100	25 µl 100 µl
19S Subunit Rpt3 (S6b), pAb	Human, mouse, rat, cow	IHC, WB	BML-PW8175-0025 BML-PW8175-0100	25 µl 100 µl
19S Subunit Rpt3 (S6b), mAb [clone TBP7-27]	Human, rabbit	IHC, IP, WB	BML-PW8765-0025 BML-PW8765-0100	25 µl 100 µl
19S Subunit Rpt4 (S10b), pAb	Human, yeast	IHC, WB	BML-PW8220-0025 BML-PW8220-0100	25 µl 100 µl
19S Subunit Rpt4 (S10b), mAb [clone p42-23]	Human, mouse	WB	BML-PW8830-0025 BML-PW8830-0100	25 µl 100 µl
19S Subunit Rpt5 (S6a), pAb	Yeast	WB	BML-PW8245-0025 BML-PW8245-0100	25 µl 100 µl
19S Subunit Rpt5 (S6a), pAb	*Arabidopsis*, cauli-flower	IHC, WB	BML-PW8375-0025 BML-PW8375-0100	25 µl 100 µl
19S Subunit Rpt5 (S6a), pAb	Human	WB	BML-PW8310-0025 BML-PW8310-0100	25 µl 100 µl
19S Subunit Rpt5 (S6a), mAb [clone TBP1-19]	Human, mouse, rat, rabbit	IHC, WB	BML-PW8770-0025 BML-PW8770-0100	25 µl 100 µl
19S Subunit Rpt6 (S8), pAb	Human, yeast	IHC, WB	BML-PW8215-0025 BML-PW8215-0100	25 µl 100 µl

Product	Specificity	Application	Prod. No.	Size
19S Subunit Rpt6 (S8), pAb	Human	WB	BML-PW8320-0025 BML-PW8320-0100	25 µl 100 µl
19S Subunit Rpt6 (S8), mAb [clone p45-110]	Human, mouse, rat	IHC, IP, WB	BML-PW9265-0025 BML-PW9265-0100	25 µl 100 µl
ATPase Subunit antibodies, sampler pack (human)			BML-PW8935-0001	10 x 10 µl
ATPase Subunit antibodies, sampler pack (yeast)			BML-PW8940-0001	8 x 10 µl

19S Regulator non-ATPase Subunits – Protein

Product	Detail/Use	Prod. No.	Size
Gankyrin, His_6-tagged	A proteasome-interacting protein	BML-UW9815-0100	100 µg

19S Regulator non-ATPase Subunits – Antibodies

Product	Specificity	Application	Prod. No.	Size
19S Subunit Rpn2 (S1), mAb [clone 112-1]	Human	WB	BML-PW9270-0025 BML-PW9270-0100	25 µl 100 µl
19S Subunit Rpn5, pAb	*S. cervisiae*, *A. thaliana*	WB	BML-PW0450-0025 BML-PW0450-0100	25 µl 100 µl
19S Subunit Rpn6 (S9), pAb	*Arabidopsis*, *cauliflower*	WB	BML-PW8370-0025 BML-PW8370-0100	25 µl 100 µl
19S Subunit Rpn7 (S10a), pAb	Human, yeast	IP, WB	BML-PW8225-0025 BML-PW8225-0100	25 µl 100 µl
19S Subunit Rpn8 (S12), pAb	Human	IHC, WB	BML-PW8180-0025 BML-PW8180-0100	25 µl 100 µl
19S Subunit Rpn10 (S5a), mAb [clone S5a-18]	Human	IHC, IP, WB	BML-PW9250-0025 BML-PW9250-0100	25 µl 100 µl
19S Subunit Rpn11 (S13), pAb	Human	WB	BML-PW9625-0025 BML-PW9625-0100	25 µl 100 µl
19S Subunit Rpn12 (S14), pAb	Human	IHC, WB	BML-PW8815-0025 BML-PW8815-0100	25 µl 100 µl
19S Subunit Rpn12 (S14), mAb [clone p31-27]	Human	IHC, WB	BML-PW8835-0025 BML-PW8835-0100	25 µl 100 µl
19S Subunit Rpn12 (S14), mAb [clone p31-38]	Human	IP	BML-PW9260-0025 BML-PW9260-0100	25 µl 100 µl
19S Subunit Rpn12 (S14), pAb	*Arabidopsis*, cauliflower	WB	BML-PW0440-0025 BML-PW0440-0100	25 µl 100 µl
Non-ATPase subunit antibodies, sampler pack (human)			BML-PW8965-0001	7 x 10 µl
ADRM1, pAb	Human, mouse	WB	BML-PW9910-0025 BML-PW9910-0100	25 µl 100 µl
Gankyrin, pAb	Human	WB	BML-PW8325-0025 BML-PW8325-0100	25 µl 100 µl

20S Proteasome Complex – Proteins

Product	Detail/Use	Prod. No.	Size
20S Proteasome complex	Isolated and purified from human erythrocytes	BML-PW8720-0050	50 µg
20S Proteasome complex	Purified from *Saccharomyces cerevisiae*	BML-PW8775-0050	50 µg
20S Immunoproteasome complex	Isolated from human spleen	BML-PW9645-0050	50 µg

Proteasome-associated Proteins – Antibodies

Product	Specificity	Application	Prod. No.	Size
PI31, pAb	Human, mouse	WB	BML-PW9710-0025 BML-PW9710-0100	25 µl 100 µl
PAC1, mAb [clone EX-5]	Human	IP, WB	BML-PW0480-0025 BML-PW0480-0100	25 µl 100 µl
PAC2, mAb [clone EX-6]	Human	IP, WB	BML-PW0485-0025 BML-PW0485-0100	25 µl 100 µl
PAC3, mAb [clone EX-7]	Human	IP, WB	BML-PW0490-0025 BML-PW0490-0100	25 µl 100 µl
PBA1, pAb	*Arabidopsis*	WB	BML-PW0430-0025 BML-PW0430-0100	25 µl 100 µl
PBF1, pAb	*Arabidopsis*	WB	BML-PW0435-0025 BML-PW0435-0100	25 µl 100 µl

20S Proteasome Assay Kits

Product	Detail/Use	Prod. No.	Size
20S Proteasome assay kit for drug discovery	Fluorogenic, non-radioactive assay for screening inhibitors and modulators of the 20S proteasome	BML-AK740-0001	1 Kit

Proteasome ELISA Kit

BML-PW0575-0001 1 Kit

Proteasomes are non-lysosomal proteolytic complexes localised primarily in the cytoplasm and in the nucleus of eukaryotic cells [1]. In patients suffering from autoimmune diseases, malignant myelo-proliferative syndromes, multiple myeloma, acute and chronic lymphatic leukaemia, solid tumour, sepsis or trauma, the concentration of circulating proteasome has been found to be elevated, to correlate with the disease state, and may have prognostic significance [1-4].

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. 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.

Potential utilisation:

- 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

LITERATURE REFERENCES:

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

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

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

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

20S Proteasome α-Subunits – Antibodies

Product	Specificity	Application	Prod. No.	Size
20S Subunits α1, 2, 3, 5, 6 & 7, mAb [clone MCP231]	Human, rabbit, rat, mouse, yeast, potato	IHC, WB	BML-PW8195-0025 BML-PW8195-0100	25 µl 100 µl
20S Subunit α2, mAb [clone MCP21]	Human, rabbit, cow	IHC, IP, WB	BML-PW8105-0025 BML-PW8105-0100	25 µl 100 µl
20S Subunit α2, mAb [clone MCP21], agarose conjugate	Human, rabbit, cow	WB	BML-PW8335-0500	0.5 ml
20S Subunit α2, mAb [clone MCP236]	Human, mouse	WB	BML-PW9385-0025 BML-PW9385-0100	25 µl 100 µl
20S Subunit α3, mAb [clone MCP257]	Human, mouse, rat, rabbit	IHC, WB	BML-PW8115-0025 BML-PW8115-0100	25 µl 100 µl
20S Subunit α4, mAb [clone MCP34]	Human	IHC, IP, WB	BML-PW8120-0025 BML-PW8120-0100	25 µl 100 µl
20S Subunit α4, mAb [clone MCP34], agarose conjugate	Human	WB	BML-PW9005-0500	0.5 ml
20S Subunit α4, mAb [clone MCP79]	Human, mouse	IHC, IP, WB	BML-PW9140-0025 BML-PW9140-0100	25 µl 100 µl
20S Subunit α5, mAb [clone MCP196]	Human, mouse, rat, rabbit	IHC, WB	BML-PW8125-0025 BML-PW8125-0100	25 µl 100 µl
20S Subunit α6, mAb [clone MCP20]	Human, rabbit	IHC, IP, WB	BML-PW8100-0025 BML-PW8100-0100	25 µl 100 µl
20S Subunit α6, mAb [clone MCP106]	Human, mouse, rabbit	WB	BML-PW9390-0001	1 ml
20S Subunit α7, mAb [clone MCP72]	Human, rat, rabbit, yeast, arthropod	IHC, WB	BML-PW8110-0025 BML-PW8110-0100	25 µl 100 µl
20S Subunit α (specificity unknown), mAb [clone HP810]	Human	IHC, IP	BML-PW8265-0100	100 µg
20S Subunit α (specificity unknown), mAb [clone HP903]	Human	IHC, IP	BML-PW8270-0100	100 µg
20S Subunit α (specificity unknown), mAb [clone HP103]	Human	ICC	BML-PW8275-0100	100 µg
20S Subunit α (specificity unknown), mAb [clone HP305]	Human	IHC	BML-PW8280-0100	100 µg
20S Proteasome 'core' subunits, pAb	Human, mouse, rat, rabbit, yeast	IHC, IP, WB	BML-PW8155-0025 BML-PW8155-0100	25 µl 100 µl
20S Proteasome 'core' subunits (yeast), pAb	Yeast	WB	BML-PW9355-0025 BML-PW9355-0100	25 µl 100 µl
20S α-Subunits antibody, sampler pack			BML-PW8900-0001	8 x 10 µl
20S α-Subunits antibody, sampler pack (for immunofluorescence)			BML-PW8925-0001	4 x 25 µg

20S Proteasome β-Subunits – Antibodies

Product	Specificity	Application	Prod. No.	Size
20S Subunit β1, mAb [clone MCP421]	Human, rabbit, yeast	IHC, WB	BML-PW8140-0025 BML-PW8140-0100	25 µl 100 µl
20S Subunit β1i, pAb	Human	IHC, WB	BML-PW8345-0025 BML-PW8345-0100	25 µl 100 µl
20S Subunit β1i, pAb	Human, mouse, rat	IHC, WB	BML-PW8205-0025 BML-PW8205-0100	25 µl 100 µl
20S Subunit β1i, mAb [clone LMP2-13]	Human, rat	IHC, WB	BML-PW8840-0025 BML-PW8840-0100	25 µl 100 µl
20S Subunit β2, mAb [clone MCP168]	Human, yeast	IHC, WB	BML-PW8145-0025 BML-PW8145-0100	25 µl 100 µl
20S Subunit β2, mAb [clone MCP165]	Human, mouse	IHC, WB	BML-PW9300-0025 BML-PW9300-0100	25 µl 100 µl
20S Subunit β2i, pAb	Human	WB	BML-PW8350-0025 BML-PW8350-0100	25 µl 100 µl
20S Subunit β2i, pAb	Human, mouse	IHC, WB	BML-PW8150-0025 BML-PW8150-0100	25 µl 100 µl
20S Subunit β2/β2i, pAb	Human, mouse, yeast	IHC, WB	BML-PW8210-0025 BML-PW8210-0100	25 µl 100 µl
20S Subunit β3, mAb [clone MCP102]	Human, rabbit, rat, mouse	IHC, WB	BML-PW8130-0025 BML-PW8130-0100	25 µl 100 µl
20S Subunit β4, pAb	Human, mouse, rat	WB	BML-PW8890-0025 BML-PW8890-0100	25 µl 100 µl
20S Subunit β5, pAb	Human	IHC, WB	BML-PW8895-0025 BML-PW8895-0100	25 µl 100 µl
20S Subunit β5i, pAb	Human	IHC, WB	BML-PW8355-0025 BML-PW8355-0100	25 µl 100 µl
20S Subunit β5i, pAb	Human, mouse, rat	IHC, WB	BML-PW8200-0025 BML-PW8200-0100	25 µl 100 µl
20S Subunit β5i, mAb [clone LMP7-1]	Human, rat	IHC, WB	BML-PW8845-0025 BML-PW8845-0100	25 µl 100 µl
20S Subunit β6, pAb	Human, rat, mouse	IHC, WB	BML-PW9000-0025 BML-PW9000-0100	25 µl 100 µl
20S Subunit β7, mAb [clone MCP205]	Human, rabbit	IHC, WB	BML-PW8135-0025 BML-PW8135-0100	25 µl 100 µl
20S Subunit β7, mAb [clone MCP219]	Human, mouse	WB	BML-PW9395-0025 BML-PW9395-0100	25 µl 100 µl
20S Subunit β7, mAb [clone MCP444]	Human	IP, WB	BML-PW9150-0025 BML-PW9150-0100	25 µl 100 µl
20S Proteasome 'core' subunits, pAb	Human, mouse, rat	IHC, IP, WB	BML-PW8155-0025 BML-PW8155-0100	25 µl 100 µl
20S Proteasome 'core' subunits (yeast), pAb	*Saccharomyces cerevisiae*	WB	BML-PW9355-0025 BML-PW9355-0100	25 µl 100 µl
20S β-Subunits antibody, sampler pack			BML-PW8905-0001	13 x 10 µl

Proteasome Inhibitors

Product	Chymotrypsin-like	Trypsin-like	Caspase-like	Prod. No.	Size
Ac-Ala-Pro-Nle-Asp-H			x	BML-AW9485-0100	100 µg
Ac-Leu-Leu-Met-H (ALLM)	x		x	BML-PI100-0005 BML-PI100-0025	5 mg 25 mg
Ac-Leu-Leu-Nle-H (ALLN)	x		x	BML-P120-0005 BML-P120-0025	5 mg 25 mg
Aclacinomycin A (Aclarubicin)	x			BML-AW8655-0005	5 mg
Ada-$(Ahx)_3$-$(Leu)_3$-vinylsulfone	x	x	x	BML-AW9155-0100	100 µg
Ada-Lys(biotinyl)-$(Ahx)_3$-$(Leu)_3$-vinyl-sulfone	x	x	x	BML-AW9165-0100	100 µg
Ada-Tyr-$(Ahx)_3$-$(Leu)_3$-vinylsulfone	x	x	x	BML-AW9160-0100	100 µg
Bactenecin-5	x	x	x	BML-BW9315-0100	100 µg
Celastrol	x			ALX-350-332-M005 ALX-350-332-M025	5 mg 25 mg
(-)-Epigallocatechin gallate (EGCG)	x			ALX-270-263-M010 ALX-270-263-M050	10 mg 50 mg
Epoxomicin	x			BML-PI127-0100	100 µg
Gliotoxin	x			BML-PI129-0002 BML-PI129-0010	2 mg 10 mg
Lactacystin (native)	x	x		ALX-350-245-MC01 ALX-350-245-MC05 ALX-350-245-M001	0.1 mg 0.5 mg 1 mg
Lactacystin (synthetic)	x	x		BML-PI104-0200 BML-PI104-1000	200 µg 1 mg
clasto-Lactacystin β-lactone	x	x		BML-PI108-0100	100 µg
NIP-$(Leu)_3$-vinylsulfone	x	x	x	BML-NW8780-0500	500 µg
N-Tosyl-Lys-chloromethylketone (TLCK)		x		BML-PI121-0200	200 mg
PR11	x	x	x	BML-PW9325-0100	100 µg
PR26	x	x	x	BML-PW9790-0100	100 µg
PR39	x	x	x	BML-PW8850-0100	100 µg
Z-Ile-Glu(OtBu)-Ala-Leu-H (PSI)	x		x	BML-ZW8410-0005	5 mg
Z-Leu-Leu-Leu-$B(OH)_2$ (MG262)	x		x	BML-PI109-0100	100 µg
Z-Leu-Leu-Leu-H (MG132)	x		x	BML-PI102-0005 BML-PI102-0025	5 mg 25 mg
Z-Leu-Leu-Leu-vinylsulfone	x	x	x	BML-ZW9170-0500	500 µg
Z-Leu-Leu-Nva-H (MG115)	x		x	BML-ZW8445-0005	5 mg
Z-Leu-Leu-Phe-H	x			ALX-260-090-M001 ALX-260-090-M005	1 mg 5 mg
Z-Leu-Leu-Tyr-ketoaldehyde	x			BML-ZW8655-0005	5 mg
Z-Pro-Nle-Asp-H			x	BML-ZW9490-0100	100 µg
Proteasome inhibitor pack	x	x	x	BML-PW9901-0001	1 Pack

Proteasome Substrates

Product	Chymotrypsin-like	Trypsin-like	Caspase-like	Prod. No.	Size
Ac-Arg-Leu-Arg-AMC		x		BML-AW9785-0005	5 mg
Ac-Gly-Pro-Leu-Asp-AMC			x	BML-AW9560-0005	5 mg
Ac-Nle-Pro-Nle-Asp-AMC			x	BML-AW9555-0005	5 mg
Boc-Leu-Arg-Arg-AMC		x		BML-BW8515-0005	5 mg
Bz-Val-Gly-Arg-AMC		x		BML-BW9375-0005	5 mg
MCMV pp89 substrate peptide	x	x	x	BML-PW9380-0100	100 µg
Suc-Arg-Pro-Phe-His-Leu-Leu-Val-Tyr-AMC	x			BML-SW8525-0005	5 mg
Suc-Leu-Leu-Val-Tyr-AMC	x			BML-P802-0005	5 mg
Suc-Leu-Tyr-AMC	x			BML-P130-0020	20 mg
Z-Leu-Leu-Glu-AMC			x	BML-ZW9345-0005	5 mg
Z-Leu-Leu-Glu-βNA			x	BML-ZW8520-0005	5 mg
Z-Gly-Gly-Leu-AMC	x			BML-ZW8505-0005	5 mg
Z-Gly-Gly-Leu-βNA	x			BML-ZW8510-0005	5 mg
Z-Leu-Leu-Leu-AMC (Proteasome Substrate I)	x			ALX-260-088-M001 ALX-260-088-M005	1 mg 5 mg
Z-Val-Lys-Met-AMC (Proteasome Substrate IV)	x			ALX-260-087-M001 ALX-260-087-M005	1 mg 5 mg
Proteasome substrate pack	x	x	x	BML-PW9905-0001	1 Pack

26S Proteasome Proteins & Kits

Product	Detail/Use	Prod. No.	Size
26S Proteasome complex	Highly purified preparation of '26S' proteasomes useful for carrying out *in vitro* protein degradation studies with suitably ubiquitinylated protein substrates.	BML-PW9310-0050	50 µg
26S Proteasome degradation kit	This kit contains a highly purified, human erythrocyte derived, preparation of '26S' proteasomes useful for carrying out *in vitro* protein degradation studies with suitably ubiquitinylated protein substrates. The preparation consists of a high purity mixture of '26S' proteasomes singly (26S) and doubly (30S) capped with 19S 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 assays.	BML-PW8950-0001	1 Kit
Proteasome ELISA Kit	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. This kit provides sufficient material for a single 96 well plate.	BML-PW0575-0001	1 Kit

COP9 Signalosome CSN – Protein

Product	Detail/Use	Prod. No.	Size
COP9 Signalosome complex	Isolated from human erythrocytes	BML-PW9425-0010	10 µg

COP9 Signalosome CSN – Antibodies

Product	Specificity	Application	Prod. No.	Size
Csn1, pAb	Human, mouse, pig	IHC, IP, WB	BML-PW8285-0025 BML-PW8285-0100	25 µl 100 µl
Csn2, pAb	Human	IHC, IP, WB	BML-PW8230-0025 BML-PW8230-0100	25 µl 100 µl
Csn2, pAb	Mouse	IP, WB	BML-PW9720-0025 BML-PW9720-0100	25 µl 100 µl
Csn3, pAb	Human	IHC, WB	BML-PW8235-0025 BML-PW8235-0100	25 µl 100 µl
Csn4, pAb	Human, *Arabidopsis*, cauliflower	WB	BML-PW8360-0025 BML-PW8360-0100	25 µl 100 µl
Csn5, pAb	*Arabidopsis*, cauliflower	WB	BML-PW8365-0025 BML-PW8365-0100	25 µl 100 µl
Csn6, pAb	Human	WB	BML-PW8295-0025 BML-PW8295-0100	25 µl 100 µl
Csn7, pAb	Human	IP, WB	BML-PW8300-0025 BML-PW8300-0100	25 µl 100 µl
Csn8, pAb	Human, mouse, pig, *Xenopus*	IHC, IP, WB	BML-PW8290-0025 BML-PW8290-0100	25 µl 100 µl
COP9 Signalosome subunit antibodies, sampler pack			BML-PW8945-0001	8 x 10 µl

TPPII – Protein

Product	Detail/Use	Prod. No.	Size
Tripeptidyl peptidase II (TPPII) complex	Isolated from human erythrocytes	BML-PW9660-0010	10 µg

TPPII – Antibody

Product	Specificity	Application	Prod. No.	Size
Tripeptidyl Peptidase II, pAb	Human	WB	BML-PW0690-0025 BML-PW0690-0100	25 µl 100 µl

p97

Product	Specificity	Application	Prod. No.	Size
VCP/p97/cdc48, pAb	Human, mouse, rat, pig	WB	BML-PW9335-0025 BML-PW9335-0100	25 µl 100 µl

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