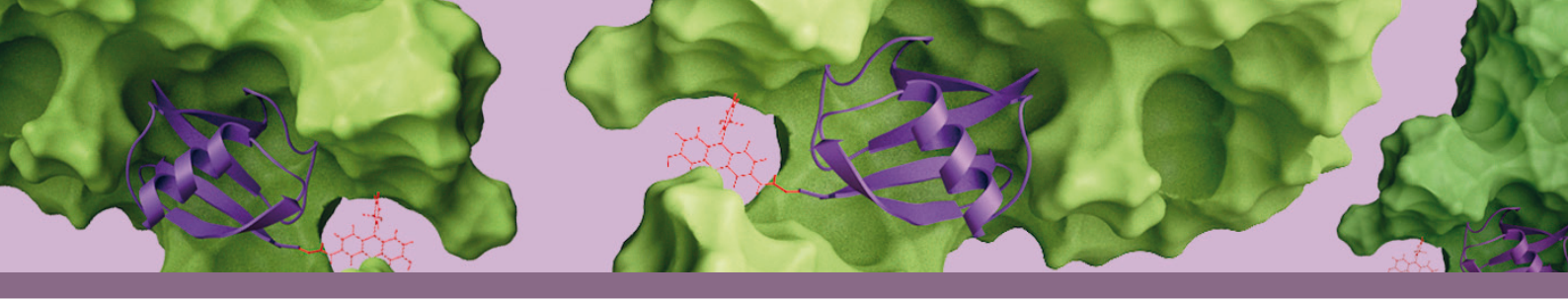


South Bay Bío

Platform Technology Company specialized in Bioassays, Enzymes and Advanced TR-FRET Technology

COLLABORATING WITH
AdipoGen®
LIFE SCIENCES

South Bay Bio, LLC, formed in 2016, provides expertise in the ubiquitin proteasome system (UPS) and other innovative research areas, like UPS related assay development, HTS, protein purification, bioconjugation and custom biochemistry services.

Product Highlights UNIQUE

Protein-based Substrates

ISG15 (human) (rec.) (Rhodamine 110)
NEDD8 (human) (rec.) (Rhodamine 110)
SUMO1 (human) (rec.) (Rhodamine 110)
SUMO2 (human) (rec.) (Rhodamine 110)

High Quality – Purified from PBMCs

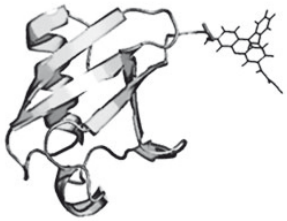
20S Immunoproteasome (human) (untagged)

Cryptate-based TR-FRET Reagents

Ubiquitin (human) (rec.) (Europium-Cryptate)
E3 Ligase TR-FRET Assays

PLEASE CONTACT US FOR SERVICES:

Protein/Enzyme Production
Enzyme Activity Determination
Assay Development (HTS/TR-FRET)
Antibody Conjugation
Protein Labeling & Purification
Europium (and Terbium) Cryptates

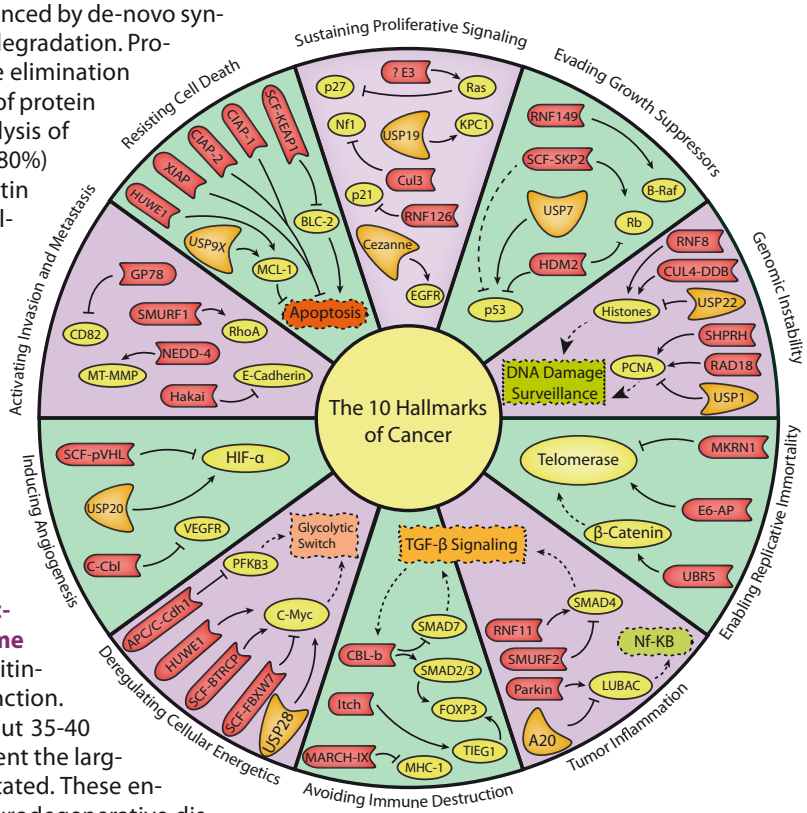


For all Products: www.adipogen.com

Contents

The Ubiquitin-Proteasome System

Protein homeostasis at the cellular level is precisely balanced by de-novo synthesis, posttranslational modifications and proteolytic degradation. Protein degradation serves several functions including the elimination of damaged and no longer needed proteins, activation of protein precursors by partial hydrolysis or the complete hydrolysis of proteins that regulate multiple functions. The majority (>80%) of all mammalian proteins are degraded by the ubiquitin proteasome system (UPS). Over the past decade, knowledge about the UPS has exploded. It has now become obvious that the ubiquitination of proteins does not solely target them for degradation, but can also play a role in regulatory functions including translational regulation, activation of transcription factors and kinases, DNA repair, endocystosis and vesicular transport of membrane proteins.



Ubiquitin Conjugation & Deconjugation

The ubiquitin conjugation system is hierarchically structured in three distinct classes of enzymes; i) **ubiquitin activating enzyme (E1)**, ii) **ubiquitin conjugating enzyme (E2)**, and iii) **ubiquitin ligases (E3)**. Only one E1 ubiquitin-activating enzyme exists highlighting its essential function. E2 conjugating enzymes are more abundant with about 35-40 discovered and characterized to date. E3 ligases represent the largest class of enzymes to date with more then 700 annotated. These enzymes have very strong implications in cancer and neurodegenerative diseases. **Deconjugating enzymes (DUBs)** catalyze the deconjugation of ubiquitin from substrate proteins. This reverses the conjugation cascade, affecting critical events in the cell proteome such as protein degradation through the proteasome. To date, more then 100 DUBs have been annotated and many studies have shown their multiple functions in human disease.

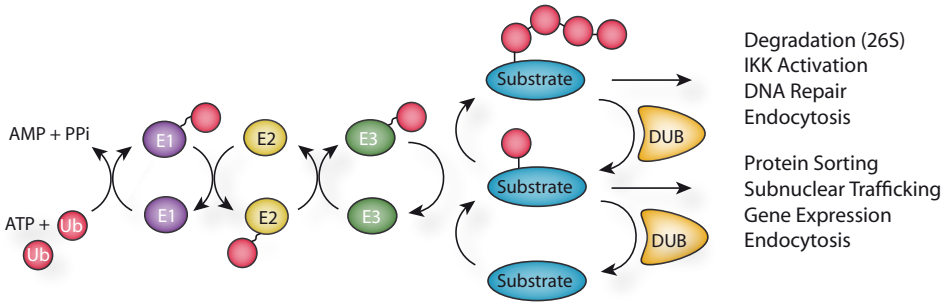


FIGURE 2: The ubiquitin conjugation system.

SELECTED REVIEW ARTICLES

Nonproteolytic functions of ubiquitin in cell signaling: Z.J. Chen & L.J. Sun; Mol. Cell **33,** 275 (2009) • The amazing ubiquitin-proteasome system: structural components and implication in aging: E.N. Tsakiri & I.P. Trougakos; Int. Rev. Cell Mol. Biol. **314**, 171 (2015) • The increasing complexity of the ubiquitin code: R. Yau & M. Rape; Nat. Cell Biol. **18,** 579 (2016) • Ubiquitin modifications: K.N. Swatek & D. Komander; Cell Res. **26,** 399 (2016) • Ubiquitin signaling in immune responses: H. Hu & S.C. Sun; Cell Res. **26,** 457 (2016)

E1 Activating Enzymes & E2 Conjugating Enzymes

PRODUCT NAME	PID	TYPE	SIZE
Ubiquitin Activating Enzyme E1 [UBA1] (human) (rec.) (untagged)	SBB-CE0011	E1	50 µg
UBE2D3 [UbcH5c] (human) (rec.) (untagged)	SBB-CE0019	E2	100 µg
UBE2L3 [UbcH7] (human) (rec.) (untagged)	SBB-CE0020	E2	100 µg
UBE2D1 [UbcH5a] (human) (rec.) (untagged)	SBB-CE0021	E2	100 µg
UBE2K [UbcH1; E2-25K] (human) (rec.) (His)	SBB-CE0022	E2	100 µg
UBE2D2 [UbcH5b] (human) (rec.) (untagged)	SBB-CE0027	E2	100 µg

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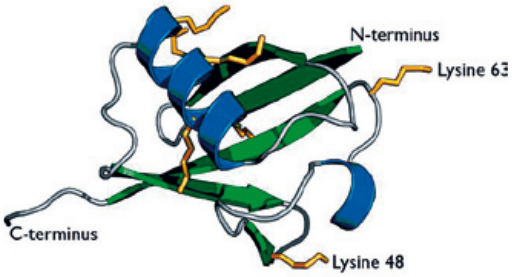
Ubiquitin (Ub) & Ubiquitin-like Proteins (UBLs)

Ubiquitin is a 76aa post-translational modifier expressed throughout all tissues in eukaryotic organisms. The many roles of ubiquitin modification include proteasomal degradation, signal transduction, inflammatory response and DNA damage repair.



Although ubiquitin is the most-understood post-translation modifier, there is a growing family of ubiquitin-like proteins (UBLs) that modify cellular targets in a pathway that is parallel to, but distinct from, that of ubiquitin. Known UBLs include: **small ubiquitin-like modifier (SUMO), interferon-stimulated gene-15 (ISG15), ubiquitin-related modifier-1 (URM1), neuronal-precursor-cell-expressed developmentally downregulated protein-8 (NEDD8), human leukocyte antigen F-associated (FAT10), autophagy-8 (ATG8) and -12 (ATG12), few ubiquitin-like protein (FUB1), MUB (membrane-anchored UBL), ubiquitin fold-modifier-1 (UFM1) and ubiquitin-like protein-5 (UBL5).** Whilst these proteins share only modest primary sequence identity with ubiquitin, they are closely related three-dimensionally. UBLs have novel functions and influence diverse biological processes, however there is also cross-regulation between the various conjugation pathways, since some proteins can become modified by more than one UBL and sometimes even at the same lysine residue.

UBLs are structurally similar to ubiquitin and are processed, activated, conjugated and released from conjugates by enzymatic steps that are similar to the corresponding mechanisms for ubiquitin. UBLs are also translated with C-terminal extensions that are processed to expose the invariant C-terminal LRGG. These modifiers have their own specific E1 (activating), E2 (conjugating) and E3 (ligating) enzymes that conjugate the UBLs to intracellular targets. These conjugates can be reversed by UBL-specific isopeptidases that have similar mechanisms to that of the deubiquitinating enzymes.

SELECTED REVIEW ARTICLES

ISG15: leading a double life as a secreted molecule: D. Bogunovic, et al.; Exp. Mol. Med. **45,** e18 (2013) • Protein neddylation: beyond cullin-RING ligases: R.I. Enchev, et al.; Nat. Rev. Mol. Cell Biol. **16,** 30 (2015) • SUMO conjugation - a mechanistic view: A. Pichler, et al.; Biomol. Concepts **8,** 13 (2017) • Prokaryotic ubiquitin-like protein and its ligase/deligase enyzmes: C.L. Delley, et al.; J. Mol. Biol. **(Epub ahead of print)** (2017)

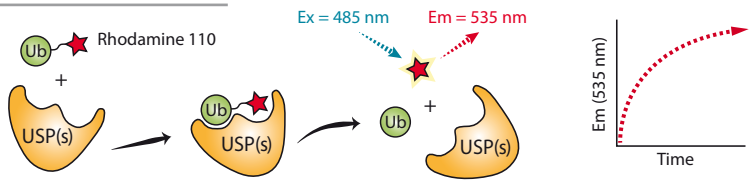
Ubiquitin (Ub) & Ubiquitinated Substrates

PRODUCT NAME	PID	SIZE
Ubiquitin (human) (rec.) (untagged)	SBB-UP0013	10 mg
p53 (polyubiquitinated) (human) (rec.) (His)	SBB-US0012	20 µg

Ubiquitin-like Proteins (UBLs) – Protein-based Substrates

PRODUCT NAME	SUBSTRATE	PID	SIZE
Ubiquitin (human) (rec.) (Rhodamine 110)	deUBIQUITINylating activity	SBB-PS0001	50 µg
ISG15 (human) (rec.) (Rhodamine 110)	deISGylating activity	SBB-PS0002	50 µg
NEDD8 (human) (rec.) (Rhodamine 110)	deNEDDylating activity	SBB-PS0003	50 µg
SUMO1 (human) (rec.) (Rhodamine 110)	deSUMOylating activity	SBB-PS0028	50 µg
SUMO2 (human) (rec.) (Rhodamine 110)	deSUMOylating activity	SBB-PS0029	50 µg

FIGURE: Scheme of protein-based substrate application.



UBL Deconjugating Enzymes – Substrates

PRODUCT NAME	SUBSTRATE	PID	SIZE
UCH-L3 (human) (rec.) (untagged)	UBIQUITINylated Proteins	SBB-DE0023	50 µg
PLpro (SARS Coronavirus) (rec.) (His)	UBIQUITINylated / ISGylated Proteins	SBB-DE0024	50 µg
NEDP1 [SENP8] (human) (rec.) (His)	NEDDylated Proteins	SBB-DE0025	50 µg
SENP1 (catalytic domain) (human) (rec.) (His)	SUMOylated Proteins	SBB-DE0026	50 µg

Active 20S Proteasome & 20S Immunoproteasome Proteins

The ubiquitin-proteasome pathway is the major proteolytic system in eukaryotic cells, where it catalyzes the selective degradation of short-lived regulatory proteins or the rapid turnover of misfolded proteins. One of the most important proteases in this pathway is the **26S proteasome**, an ATP-dependent proteolytic complex, which is formed by the association of the barrel-shaped **20S proteasome** (700kDa) and two 19S (700kDa) regulatory complexes. The 20S catalytic core is composed of 4 rings of 28 non-identical subunits; 2 rings are composed of 7 α-subunits and 2 rings are composed of 7 β-subunits. The 20S catalytic core is able to degrade a variety of peptide substrates and poly-ubiquitinated proteins involved in apoptosis, DNA repair, endocytosis and cell cycle control.

The **immunoproteasome** is structurally similar to constitutive 26S proteasome. The 20S core of immunoproteasome contains two outer rings composed of α-subunits and two internal 7-subunit containing rings each possessing 3 specific subunits responsible for proteasome catalytic activity. In the 20S immunoproteasome these subunits (β1, β2, β5) are replaced by three inducible subunits: PSMB9/LMP2, PSMB10/MECL1 and PSMB8/LMP7 (β1i, β2i, β5i). These stress-induced subunits allow for the production of MHC-1 associating peptides, which are displayed as antigens on the cell surface. These displayed peptides can then be recognized by immune surveillance CD8 T cells. The 20S immunoproteasome is recognized as a strong drug target for autoimmune disease and cancer. The 20S immunoproteasome is commonly associated with the 19S, PA28 α/β or the PA28γ regulatory complexes.

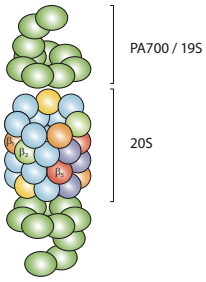


FIGURE: 26S Proteasome.

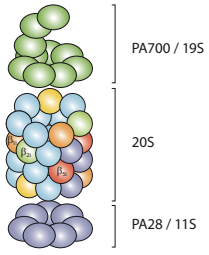


FIGURE: Immunoproteasome.

PRODUCT NAME	PID	SIZE
20S Immunoproteasome (human) (untagged)	SBB-PP0004	25 µg
20S Proteasome (human) (untagged)	SBB-PP0005	50 µg

NEW Proteasome Assays — Coming soon

PRODUCT NAME	PID	SIZE
20S Immunoproteasome Assay Kit	SBB-KP0037	1 Kit
20S Constitutive Proteasome Assay Kit	SBB-KP0038	1 Kit

SELECTED REVIEW ARTICLES

New insights into the function of the immunoproteasome in immune and non-immune cells: H. Kimura, et al.; J. Immunol. Res. **2015,** 541984 (2015) • Ubiquitin recognition by the proteasome: Y. Saeki; J. Biochem. **161,** 113 (2017) • The logic of the 26S proteasome: G.A. Collins & A.L. Goldberg; Cell. **169,** 792 (2017)

Potent Deubiquitinating Enzymes (DUBs) Inhibitors — NEW

Potent, irreversible and specific inhibitors of deubiquitinating enzymes (DUBs) that can be used for activity profiling experiments and determining DUB inhibitor specificity. These inhibitors target three of the four major DUB families: UCH (Ubiquitin C-terminal Hydrolases), USP (Ubiquitin Specific Proteases), OTU (Ovarian Tumor Proteases), and MJD (Machado-Josephin Domain Proteases) while JAMM Metalloproteases are not inhibited.

PRODUCT NAME	PID	SIZE
Ubiquitin aldehyde (human) (rec.)	SBB-PS0031	50 µg
Ubiquitin propargylamide (human) (rec.)	SBB-PS0034	50 µg
Ubiquitin vinyl methyl ester (human) (rec.)	SBB-PS0033	50 µg
Ubiquitin vinyl sulfone (human) (rec.)	SBB-PS0032	50 µg

Also Available:

PRODUCT NAME	DESCRIPTION	PID
Anacardic acid	SUMOylation inhibitor.	AG-CR1-0046
Auranofin	Proteasomal deubiquitinase inhibitor.	AG-CR1-3611
b-AP15 [DUB Inhibitor] Solution	Inhibitor of the 19S deubiquitinase (DUB), ubiquitin-specific-processing protease 14 (USP14) and ubiquitin C-terminal hydrolase isozyme L5 (UCH-L5).	AG-CS1-0102

Proteasome Inhibitors / Substrates / Probes

Although eukaryotic 20S proteasomes harbor seven different β-subunits, only three β-subunits per β-ring **[subunits β1 (caspase-like), β2 (trypsin-like) and β5 (chymotrypsin-like)]** are proteolytically active. These three β-subunits are major targets for small molecule proteasome inhibitors. The blockade or inactivation of the 26S proteasome complex-regulated degradative process, using small molecule inhibitors against one or more catalytic β-subunits, can lead to significant build-up of cytotoxic proteins. Proteasome inhibition has implications in a number of human diseases such as cancer, inflammation and ischemic stroke and is an important therapeutic target.

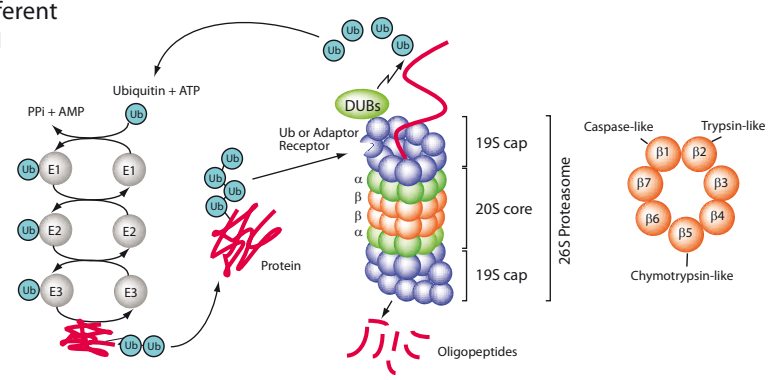


FIGURE: The ubiquitin proteasome system.

Specialized variants of the constitutive 20S proteasome in the immune system like the immunoproteasomes contain active site-bearing subunits which differ in their cleavage priorities and substrate binding pockets. The immunoproteasome plays a crucial role in antigen processing and for the differentiation of pro-inflammatory T helper cells which are involved in the pathogenesis of autoimmunity.

NEW Proteasome Inhibitors

PRODUCT NAME	DESCRIPTION	PID
Carfilzomib [PR-171]	Chymotrypsin-like (β5 +β5i subunit) activity inhibitor.	AG-CR1-3669
Ixazomib [MLN2238]	Chymotrypsin-like (β5) (IC_{50}=3.4nM), caspase-like (β1) (IC_{50}=31nM) and trypsin-like (β2) (IC_{50}=3.5μM) activity inhibitor.	AG-CR1-3670
Ixazomib citrate [MLN9708]	Chymotrypsin-like (β5) (IC_{50}=3.4nM), caspase-like (β1) (IC_{50}=31nM) and trypsin-like (β2) (IC_{50}=3.5μM) activity inhibitor.	AG-CR1-3671
Oprozomib [ONX 0912]	Chymotrypsin-like (β5) (IC_{50}=36nM) and (β5i) (IC_{50}=82nM) activity inhibitor.	AG-CR1-3672
Delanzomib [CEP-18770]	Chymotrypsin-like (β5) (IC_{50}=3.8nM) activity inhibitor.	AG-CR1-3673
ONX 0914	Chymotrypsin-like (β5i) (IC_{50}=73nM) and (β5) (IC_{50}=1.04μM) activity inhibitor.	AG-CR1-3674
PI-1840	Chymotrypsin-like (β5) (IC_{50}=27nM) activity inhibitor.	AG-CR1-3675
VR23	Trypsin-like (β2) (IC_{50}=1nM), chymotrypsin-like (β5) (IC_{50}=100nM) and caspase-like (β1) (IC_{50}=3μM) activity inhibitor.	AG-CR1-3676

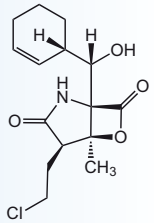
Salinosporamide A – A Potent 20S Proteasome Inhibitor

NEW

Salinosporamide A [SalA; Marizomib; NPI-0052; ML858]

AG-CN2-0444-C100 100 μg

Formula: $C_{15}H_{20}ClNO_4$ **MW:** 313.8 **CAS:** 437742-34-2



Potent, irreversible inhibitor of all the three proteolytic activities of the mammalian 20S proteasome.
- β5-subunit: chymotrypsin-like (EC_{50}=3.5nM)
- β2-subunit: trypsin-like (EC_{50}=28nM)
- β1-subunit: caspase-like or peptidyl-glutamyl peptide-hydrolyzing (PGPH) (EC_{50}=430nM)

LIT: Salinosporamide A: a highly cytotoxic proteasome inhibitor from a novel microbial source, a marine bacterium of the new genus salinospora: R.H. Feling, et al.; Angew. Chem. Int. Ed. Engl. **42,** 355 (2003) • Discovery and development of the anticancer agent salinosporamide A (NPI-0052): W. Fenical, et al.; Bioorg. Med. Chem. **17,** 2175 (2009) • Salinosporamide natural products: Potent 20S proteasome inhibitors as promising cancer chemotherapeutics: T.A. Gulder & B.S. Moore; Angew. Chem. Int. Ed. Engl. **49,** 9346 (2010) (Review) • Marizomib, a proteasome inhibitor for all seasons: preclinical profile and a framework for clinical trials: B.C. Potts, et al.; Curr. Cancer Drug Targets **11,** 254 (2011) (Review) • Antileukemic activity and mechanism of drug resistance to the marine Salinispora tropica proteasome inhibitor salinosporamide A (Marizomib): D. Niewerth, et al.; Mol. Pharmacol. **86,** 12 (2014) • Marizomib, a potent second generation proteasome inhibitor from natural origin: L. Ma & A. Diao; Anticancer Agents Med. Chem. **15,** 298 (2015) (Review) • Induction of cell death by the novel proteasome inhibitor marizomib in glioblastoma in vitro and in vivo: C.A. Manton, et al.; Sci. Rep. **6,** 18953 (2016) • Marizomib for central nervous system-multiple myeloma: A. Badros, et al.; Br. J. Haematol. **177,** 221 (2017)

Standard Proteasome Inhibitors – From the Source!

PRODUCT NAME	DESCRIPTION	PID
Bortezomib [PS-341]	Chymotrypsin-like (β5) and caspase-like (β1) activity inhibitor.	AG-CR1-3602
Epoxomicin	Predominant chymotrypsin-like (β5) activity inhibitor.	AG-CN2-0422
clasto-Lactacystin β-lactone	Chymotrypsin-like (β5), trypsin-like (β2) & caspase-like (β1) activity inhibitor.	AG-CN2-0442
Lactacystin	Chymotrypsin-like (β5), trypsin-like (β2) & caspase-like (β1) activity inhibitor.	AG-CN2-0104
Z-Leu-Leu-Phe-CHO [MG-110]	Chymotrypsin-like (β5) activity inhibitor.	AG-CP3-0021
Z-Leu-Leu-Nva-CHO [MG-115]	Chymotrypsin-like (β5) activity inhibitor.	AG-CP3-0015
Z-Leu-Leu-Leu-CHO [MG-132]	Chymotrypsin-like (β5) and caspase-like (β1) activity inhibitor.	AG-CP3-0011
Z-Leu-Leu-Leu-B(OH)$_2$ [MG-262]	Chymotrypsin-like (β5) and caspase-like (β1) activity inhibitor.	AG-CP3-0024

Natural Product Proteasome Inhibitors

Celastrol	Chymotrypsin-like (β5) (IC_{50}=2.5μM) activity inhibitor.	AG-CN2-0460
(-)-Epigallocatechin gallate [EGCG]	Chymotrypsin-like (β5) activity inhibitor (IC_{50}~200nM).	AG-CN2-0063
Quercetin . dihydrate	Inhibits all three catalytic activities (IC_{50}~15μM).	AG-CN2-0409
Shikonin	Chymotrypsin-like (β5) (IC_{50}=12.5μM) activity inhibitor.	AG-CN2-0487
Withaferin A	Chymotrypsin-like (β5) (IC_{50}=4.5μM) activity inhibitor.	AG-CN2-0490

Fluorogenic Probes for Proteasome Activity Measurement

Peptide-AMC conjugates are excellent substrates for monitoring proteasome and/or immunoproteasome activity and to measure the ability of new inhibitors to block different active sites of proteasomes. These substrates are three to four amino acid residue peptides with a fluorogenic reporter group at the C terminus. The proteasome cleaves an amido bond between an amino acid and the reporter group, resulting in release of a highly fluorescent product. Three subunits on each inner ring of the proteasome carry catalytic residues for the proteolytic sites.

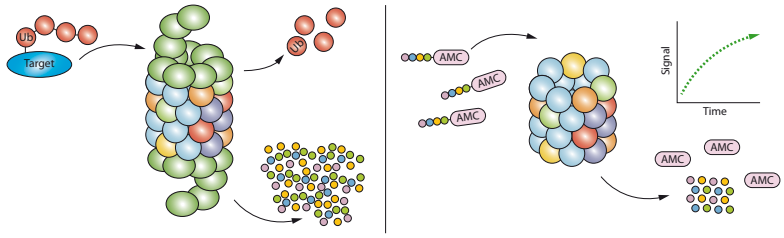


FIGURE: Schematic of proteasome use and fluorogenic substrate application.

The catalytic residues of the chymotrypsin-like sites cleave peptide bonds predominantly after hydrophobic residues. The caspase-like sites cleave peptide bonds after acidic residues. The trypsin-like sites cleave peptide bonds after basic residues. The released AMC fluorescence can be monitored using a plate reader or fluorometer at the excitation/emission wavelength of 360nm/460nm, respectively.

PRODUCT NAME	TYPE	PID
Z-Leu-Leu-Glu-AMC	Caspase-like activity of 20S Proteasome (β1-subunit).	SBB-PS0006
Ac-Pro-Ala-Leu-AMC	Caspase-like activity of 20S Immunoproteasome (β1i-subunit).	SBB-PS0007
Ac-Trp-Leu-Ala-AMC	Chymotrypsin-like activity of 20S Proteasome (β5-subunit).	SBB-PS0008
Ac-Ala-Asn-Trp-AMC	Chymotrypsin-like activity of 20S Immunoproteasome (β5i-subunit).	SBB-PS0009
Suc-Leu-Leu-Val-Tyr-AMC	Chymotrypsin-like peptidase activity of 20S and 26S proteasome, (β5-subunit) calpains and other chymotrypsin-like proteases.	SBB-PS0010
Ac-Arg-Leu-Arg-AMC	Trypsin-like activity of the 20S proteasome (β2-subunit).	AG-CP3-0013
Boc-Leu-Arg-Arg-AMC	Trypsin-like activity of the 20S proteasome (β2-subunit).	AG-CP3-0014
Suc-Leu-Leu-Val-Tyr-AMC	Chymotrypsin-like activity of the 20S proteasome (β5-subunit).	AG-CP3-0016
Suc-Leu-Tyr-AMC	Chymotrypsin-like activity of the 20S proteasome (β5-subunit).	AG-CP3-0017
Z-Leu-Leu-Leu-AMC	Chymotrypsin-like activity of the 20S proteasome (β5-subunit).	AG-CP3-0019
Z-Leu-Leu-Glu-AMC	Caspase-like activity of the 20S proteasome (β1-subunit).	AG-CP3-0022
Me$_4$BodipyFL-Ahx$_3$Leu$_3$VS	Cell permeable fluorescent proteasome activity probe.	AG-CR1-3601
Z-Leu-Arg-Gly-Gly-AMC	UCHs & Isopeptidase T substrate.	AG-CP3-0023

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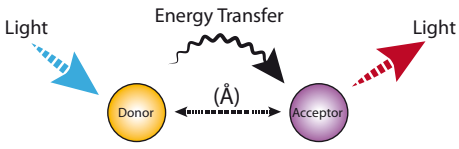
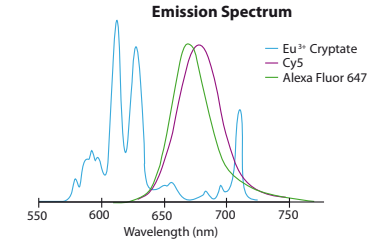
TR-FRET Measurement of Ubiquitin Conjugation & Deconjugation



Ubiquitin (Ub) is a highly conserved protein (from yeast to mammals) that plays a major role in the ubiquitination pathway. Ubiquitination, the conjugation of ubiquitin to other proteins is essential for many cellular process primarily linked to protein degradation. The ubiquitination process involves three steps with specific groups of enzymes in an ATP-dependent manner, which are activation with ubiquitin-activating enzymes (E1s), conjugation with ubiquitin-conjugating enzymes (E2s) and ligation with ubiquitin ligases (E3s).

Good FRET pairs exhibit no overlapping spectrum between donor and acceptor, high quantum yields and red-shifted emission (e.g. Cy5) to minimize compound interference. The best donors are cryptates, comprised of rare-earth complexes (europium or terbium) with a lanthanide embedded in a macrocycle. They exhibit long-lived fluorescence, stability and robustness necessary to survive different assay conditions.

Europium Cryptate-labeled Ubiquitin is ideal for measuring Ub-chain conjugation or deconjugation as a TR-FRET donor. Its ideal protein-based TR-FRET pair acceptor is **Cy5-labeled Ubiquitin.**



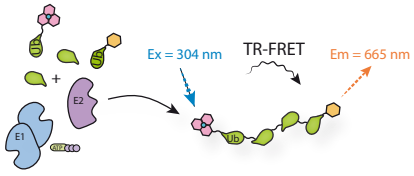


FIGURE: TR-FRET Measurement using Europium-Cryptate-labeled Ub as donor and Cy5-labeled Ub as acceptor.

Labeled Ubiquitins for TR-FRET Experiments

PRODUCT NAME	PID	EX_{MAX}	EM_{MAX}	APPLICATION	SIZE
Ubiquitin (human) (rec.) (Europium-Cryptate)	SBB-TR0014	304 nm	620 nm	Donor	20 µg
Ubiquitin (human) (rec.) (Cy5)	SBB-TR0015	646 nm	662 nm	Acceptor	50 µg

Also Available:

Ubiquitin (human) (rec.) (6-FAM)	SBB-TR0016	494 nm	520 nm	Acceptor for Terbium-Ub	50 µg

A Real-Time Homogenous TR-FRET Ubiquitin Conjugation and Deconjugation Assay Platform

South Bay Bio's homogeneous Real-Time TR-FRET ubiquitin conjugation assays are simple; the format is 96 or 384 well low-volume plates (making them well suited for HTS). Using ubiquitin either labeled with Europium-Cryptate (donor) or Cyanine 5 (acceptor), for the first time ubiquitin conjugation and deconjugation can be measured homogenously in Real-Time (facilitating enzyme kinetics or endpoint if preferred), with assays commonly exhibiting Z' 0.8 and a Signal to Noise of commonly >3000%.

Auto-ubiquitination kinetics of several human recombinant E3 ligases of significant interest, namely MDM2, MuRF1, ITCH and Parkin, along with ubiquitin conjugation on substrates such as p53 and s5a have been validated, as well as TR-FRET based deconjugation kinetics of several auto-ubiquitinated ligases using USP2cd and USP7. Coupled with the assays' short development time (as no antibody development is required for assay optimization), the assay platform is ideally suited for a wide variety of academic and industry screening applications.

Coming soon

NEW E3 Ligase TR-FRET Assays

PRODUCT NAME	PID
Mdm2 E3 Ligase TR-FRET Kit	SBB-KF0030
ITCH E3 Ligase TR-FRET Kit	SBB-KF0035
Parkin E3 Ligase TR-FRET Kit	SBB-KF0036

LITERATURE

I. Tomasic: Poster at the EMBO Conference 2017: Ubiquitin and SUMO: From molecular mechanisms to system-wide responses, in Cavtat-Dubrovnik

Technology Insights

Cryptates vs. Chelates

Rare earth cryptates are macrocyclical structures encasing a rare earth lanthanide atom. Lanthanide ions do not exhibit suitable fluorescence properties on their own and require incorporation with organic moieties designed as light-harvesting devices to collect and transfer energy by intramolecular non-radiative processes efficiently. South Bay Bio's europium cryptate is composed of a macrocycle in a cage shaped assembly composed of three bipyridine arms, which complex an Eu^{3+} ion (Eu^{3+} Trisbipyridine) as shown in the caged structure (Figure). Unlike other common flavors of rare earth complexes like chelates, cryptates are extremely robust and stable structures. This is largely due to cryptates having no dissociation between the complexed ion and the macrocycle, contrary to chelates, which often exhibit uncoordinated bonds with solvents.

Where a chelate may release its lanthanide ion in the presence of EDTA or other metal chelators, cryptates remain insensitive to these agents. These properties allow cryptates to perform in stringent conditions, e.g. in presence of chelators, of divalent cations (e.g. Mn^{2+}, Mg^{2+}), extreme pH conditions, organic solvents and high temperatures (PCR or other thermoregulated processes). Cryptates always work, Chelates don't.

COMPARISON	CRYPTATE	CHELATE
Stability	high	low
Uncoordinated Solvent Bond	0	1 to 3
EDTA / EGTA Resistant	yes	no
Divalent Cation Tolerant	yes	no
Extreme pH Condition	yes	no
High Organic Solvent Content	yes	no
High Temperature	yes	no

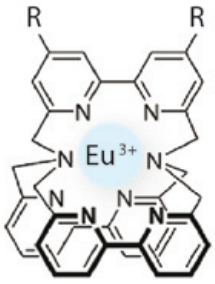


FIGURE: Eu^{3+} Trisbipyridine Structure.

LITERATURE REFERENCE

Lanthanide-based time-resolved luminescence immunoassays: A.K. Hagan & T. Zuchner; Anal. Bioanal. Chem. **400,** 2847 (2011) • Luminescent lanthanide cryptates: from the bench to the bedside: J.M. Zwier, et al.; Inorg. Chem. **53,** 1854 (2014) • HTRF: a technology tailored for biomarker determination-novel analytical detection system suitable for detection of specific autoimmune antibodies as biomarkers in nanogram level in different body fluids: L. Einhorn & K. Krapfenbauer; EPMA J. **6,** 23 (2015)

Coming soon

Cryptate-labeled & Cy5-labeled Binders

South Bay Bio develops a comprehensive selection of conjugated binders – anti-Tag antibodies and Streptavidins – for detecting a broad diversity of motifs. Features of these new binders are high-affinity (antibodies), high sensitivity (streptavidins), high resistance (to most buffer conditions and additives), compatibility with membrane- and cell-based assays, easy handling and long-term storage.

PRODUCT NAME	PID
anti-FLAG (DYKDDDDK) mAb (Europium-Cryptate)	SBB-AF0039
anti-FLAG (DYKDDDDK) mAb (Cy5)	SBB-AF0041
Streptavidin (Europium-Cryptate)	SBB-AF0040
Streptavidin (Cy5)	SBB-AF0042