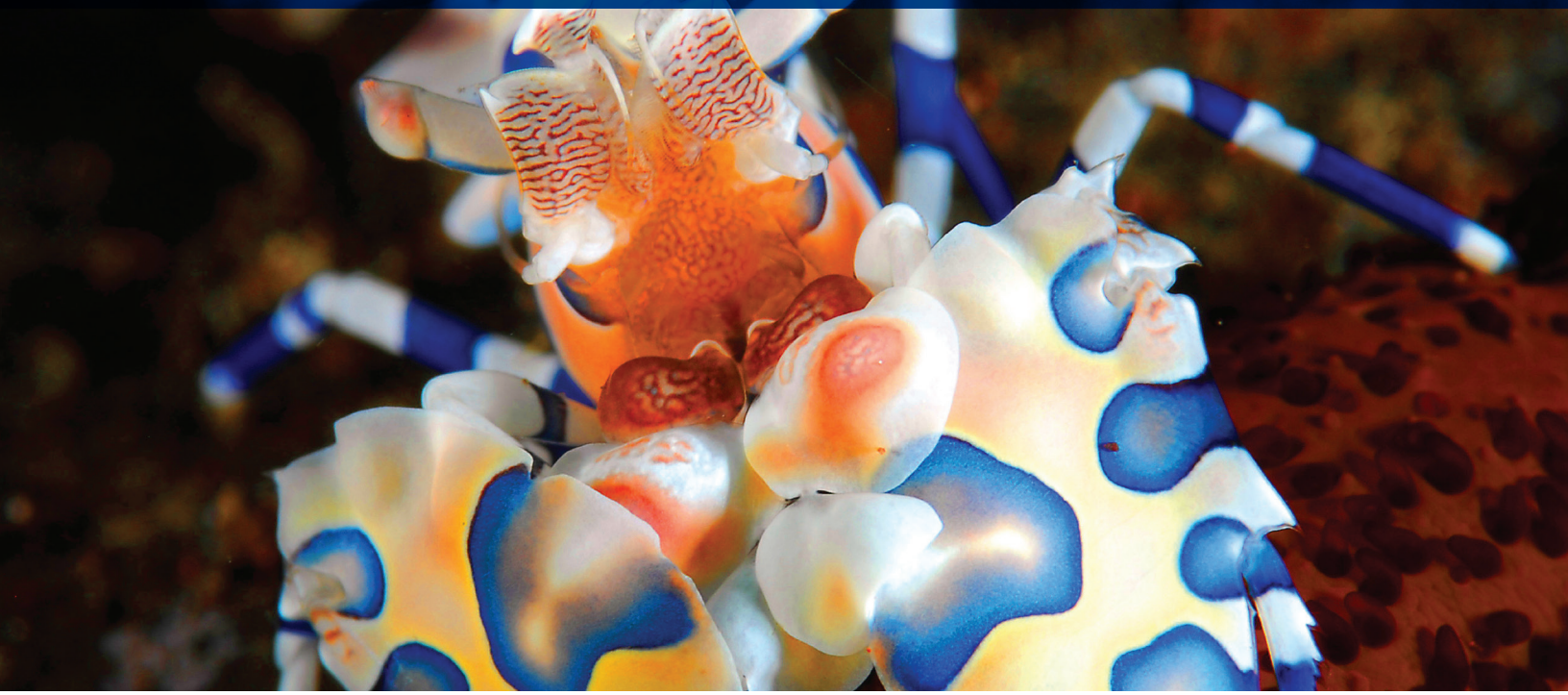
ISSUE 24
CAYMAN CURRENTS

Cayman CHEMICAL

AUTOPHAGY & THE UBIQUITIN-PROTEASOME PATHWAY



IN THIS ISSUE:

Recycling the Cell: Autophagy and the Ubiquitin-Proteasome Processes

by Paul Domanski

In order for the cell to function normally, a balance must be maintained between the production, degradation, and clearance of cytoplasmic components. To accomplish this, the cell relies primarily on two methods: 1) the ubiquitin-proteasome pathway, in which proteins are selectively tagged by ubiquitin for degradation in the proteasome and 2) autophagy ('self-eating'), a general term used to describe all pathways that are used to deliver cytoplasmic components to the lysosome for degradation. While the ubiquitin-proteasome pathway is mainly used to degrade short-lived and abnormal proteins, autophagy is responsible for elimination of cytoplasmic components, damaged organelles, and long-lived and aggregated proteins. This cellular 'recycling' system is tightly regulated so that degradation and regeneration of the cellular building blocks can proceed in an efficient manner.

Ubiquitin

Ubiquitination, one of the most common post-translational modifications (PTMs) in the cell, is the primary mechanism by which short-lived proteins are targeted for degradation and clearance. It is a highly specific system in which ubiquitin, a small (76 amino acid) protein,

continue to page 2

forms an amide bond with an epsilon amine of lysine in the target protein. This happens in a three-step enzymatic process:

1) Activation of ubiquitin by E1 (ATP-dependent).

2) Transfer of ubiquitin from E1 to the active site cysteine of a ubiquitin-conjugating (UBC) enzyme E2 (more than 30 UBC proteins are known).

3) Ligation of the C-terminal glycine of ubiquitin to the target protein by E3. The E3 family of ubiquitin ligases currently numbers more than 500 members.

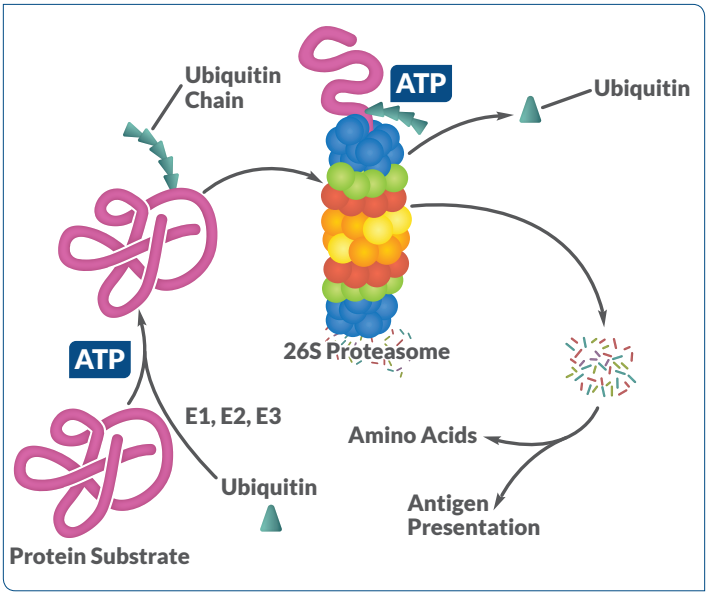


Figure 1. The Ubiquitin-Proteasome Pathway

Target proteins are ubiquitinated in a three-step ATP-dependent process involving E1, E2, and E3. The protein is then unfolded and degraded into small peptides in the proteasome, where they can be used in antigen presentation or hydrolyzed to individual amino acids.

In the ubiquitination cascade, E1 can bind with dozens of E2s, which can bind with hundreds of E3s in a hierarchical way. Furthermore, proteins can be monoubiquitinated, multiubiquitinated, and polyubiquitinated, which adds a certain depth to the pathway, as different types of linkages can activate different signaling pathways, through binding to specific ubiquitin binding domains (more than 20 have been discovered). Similar to phosphorylation, ubiquitination is reversible, and ~85 deubiquitinases (DUBs) are known. The majority of ubiquinated proteins are destined for degradation by the proteasome, however, these modifications can also target proteins to particular destinations in the cell as well as mediate protein-protein interactions **(Figure 1)**.[1-3]

Ubiquitination is involved in numerous cell functions and pathologies, including immune response, DNA repair, signal transduction, cancer, neurological disorders, and more. Obviously, any defects in the pathway can have widespread effects in the cell and are implicated in a range of disease states including neurodegeneration, cancer, and cardiovascular disease.[4,5] With the advent of new reagents and technology (*e.g.*, mass spectrometry), greater focus is placed on developing drugs that target the ubiquitin-proteasome pathway.

Cayman has an extensive product portfolio geared toward the study of ubiquitin and the proteasome. Numerous chemicals that can enhance or block the effects of ubiquitination are available to allow specific pathways in the ubiquitination process to be analyzed. We also have a panel of antibodies, including FK1 and FK2 (**see page 9**), which can be used to screen for particular types of ubiquitination of target proteins (monoubiquitination, multiubiquitination, and polyubiquitination). We have recently added kits that can be used to test for ubiquitination, ubiquitin binding proteins, and deubiquitinase activity, giving researchers key tools needed to further investigate the processes involved and to discover compounds that influence these pathways (**see pages 9-10**).

Autophagy

Autophagy is an evolutionarily conserved catabolic process in which cellular components are degraded through the lysosomal machinery. As such, it is a normal part of cell growth, development, differentiation, and homeostasis. This enables the cell to maintain a strict balance between synthesis, degradation, and recycling of cellular components. In lesser organisms, its purpose is to maintain the metabolic equilibrium with ever-changing nutrient availability.[6] Although originally thought to be a 'crude' process for the destruction of cell constituents, it has become apparent that there is a 'selective' aspect to autophagy. In higher organisms, this system can eliminate aggregated proteins, damaged organelles such as mitochondria (mitophagy) and peroxisomes (pexophagy), and can also remove microbial pathogens (xenophagy) **(Figure 2)**.[7,8]

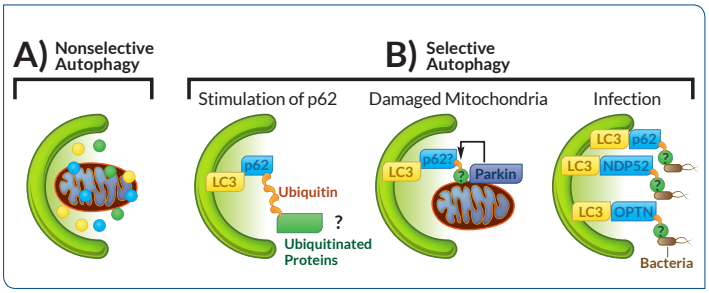


Figure 2. Nonselective and Selective Autophagy

(A) Under starvation conditions, nonselective autophagy catabolizes components in the cytoplasm into basic building blocks that the cell machinery can reuse. (B) Certain stimuli can drive selective autophagy, as in stimulation of p62, damaged mitochondria, or infection.

Autophagy is continuously operating at a basal level in cells, but is also highly inducible in response to internal and external stimuli. Numerous signaling pathways are involved in autophagy, though to what extent is not well understood. Many proteins have been implicated in the process of autophagy, including mTOR, p62, Beclin 1, and PINK1 **(Figure 3)**.[8] Due to the balancing act that autophagy must perform, one would expect that any defects in the system could play an important role in disease. Indeed, it has been shown that knockout mice lacking an essential gene for autophagy will die within hours of birth.[9] It is also known that disruptions in autophagy have a role

in cancer, neurodegenerative diseases (both Huntington's and Alzheimer's disease cells show increased autophagic vacuole accumulation), lysosomal storage diseases, infectious diseases and DNA damage response (through HDACs) and may play a role in aging and cell death (through mitophagy).[10,11]

The hallmark of autophagy is the presence of a unique organelle called the autophagosome, a double membrane-bound vacuole that can range in size from 300 to 900 nm. Autophagosomes arise from a single membrane structure known as the phagophore, which elongates and then closes in on itself, sequestering cytoplasmic material in the process. These fully enclosed phagosomes then fuse with the lysosome to form the autolysosome, which allows for degradation of the components.[6]

In the late 1990s, studies in yeast led to the elucidation of a set of more than 20 autophagy-related genes (ATGs), and further research led to the discovery of mammalian homologs for many of these genes and their associated proteins.[12,13] A schematic representation of the signaling cascade, as well as known inhibitors and activators, is shown in **Figure 3**. Under nutrient-rich conditions, the protein kinase mTOR (mammalian target of rapamycin) is a primary negative regulator of autophagy. However, in a starvation situation, mTOR is deactivated, which allows ULK (the mammalian counterpart of the yeast protein ATG1) to become activated and translocate to the endoplasmic reticulum, initiating the autophagy cascade. The formation of the phagosome concludes when ATG8 (in mammals, microtubule-associated protein light chain 3, (MAP-LC3 or LC3-I)) is cleaved in a ubiquitin-like process at the C-terminus by ATG4 followed by the addition of a phosphatidylethanolamine (PE) moiety to ATG8. This LC3-phospholipid (LC3-II) covalently associates with the phagophore and is essential for the formation of the phagosome. As such, LC3-II serves as an excellent marker for detection of the autophagosome. It is worth noting that this process, as with ubiquitination, is reversible, with ATG4 also playing a role in the delipidation reaction.

Several methods exist for analyzing the extent of autophagy in cells. Early attempts relied on measuring increases in the numbers of autophagosomes by staining with acidotropic dyes such as monodansylcadaverine (MDC) (Cayman's Autophagy/Cytotoxicity Dual Staining Kit (**see page 4**)) and transmission electron microscopy (TEM), the first detection method for autophagy.[14]

Interestingly, the phospholipid modification directed to LC3 causes an elecrophoretic mobility shift, which can be used to determine the levels of LC3-I and LC3-II by analysis on western blot. When this technique is applied in concert with known lysosomal inhibitors, such as Bafilomycin A_1, the accumulation of LC3-II in the autophagosome-lysosome can be measured, which allows one to ascertain 'autophagic flux', the rate at which proteins and cellular material are cleared through the autophagic process. This principle is the rationale behind Cayman's Autophlux Kit (**see page 4**) in which an analysis of LC3-II and p62 (a known LC3 binding protein) in concert with Bafilomycin A_1 provides researchers with the means to determine the changes in autophagic flux in a cellular system under various conditions.[14-17]

In addition, Cayman offers several chemical modulators of autophagy (**see pages 6-7**) along with a LC3 Interact Kit (**see page 5**), which can be used to identify proteins that interact with members of the autophagy pathway.

The ubiquitin-proteasome and autophagy pathways are vital to the cell's ability to maintain the delicate balance between the production and degradation of proteins and cellular components. It is now understood that these pathways can also involve 'cross-talk', and that autophagy can act as an additional method for eliminating ubiquitinated proteins under conditions of extreme stress. Although much is known, much more research must be accomplished to elucidate improved biomarkers for determining how these pathways can be utilized in relevant disease settings to address and monitor therapeutic drug effectiveness. ■

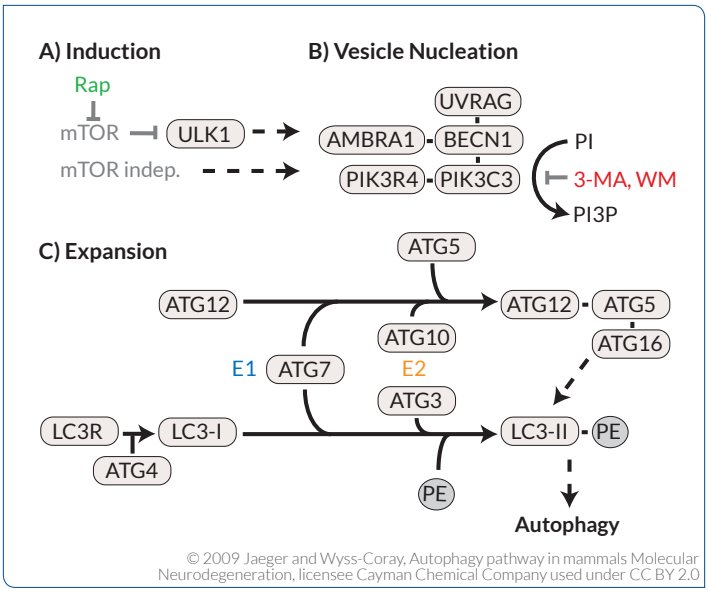


Figure 3. Autophagy Pathway in Mammals
(A) Prototypical induction of autophagy by mTOR (inhibited by rapamycin) that leads to the (B) Nucleation/expansion of the autophagosomal membrane using a protein complex known to contain Beclin 1 and PI3K (inhibited by 3-methyladenine (3-MA) and wortmannin). (C) Expansion of the membrane occurs *via* a ubiquitin like mechanism, wherein ATG7 and ATG3 convert LC3-I to LC3-II, which localizes to the autophagosome membrane.

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AUTOPHAGY DETECTION

PRODUCT SPOTLIGHT

Autophagy can be assessed by measuring the autophagic flux, which encompasses the entire process of autophagy from phagophore formation to substrate degradation and release of breakdown products. Induced changes to this system through intervention (*e.g.*, inhibitor treatment, gene knockdown, plasmid transfection, *etc.*) provide an excellent indication of overall autophagic state. The LC3-II (a lipid modified form of LC3-I) and p62 (a ubiquitin binding autophagy substrate receptor) proteins are two key biomarkers used for the measurement of autophagic flux. A comparison of levels of these proteins over time in the presence and absence of lysosomal degradation (*i.e.*, a vacuolar ATPase inhibitor) can be used to represent the amount of LC3-II or p62 degraded and, thus, indicate the rate of autophagic flux.

Using a combination of autophagy markers to assess autophagic flux offers a significant advantage of improved accuracy over the static analysis of individual biomarkers. For example, LC3-II can associate with non-autophagic membranes, and p62 can be degraded by the proteasome. Employing more than one autophagy biomarker overcomes the limitations of any one single marker for assessing such a complex system. When used together, LC3-II and p62 create a highly reliable autophagy detection system that may supplant alternative methods for autophagy detection such as the nonspecific autophagic vacuole tracer, MDC. See the comparison chart below for more details.

Special offer. See page 11

Autophlux Kit 15980

Summary: Cayman's Autophlux Kit facilitates the measurement of autophagy in cells. The kit utilizes highly characterized antibodies to key autophagy biomarkers, LC3-II and p62, together with the application of a specific, high-purity control inhibitor for the accurate assessment of autophagic flux by western blotting methods. The kit includes control cell extracts for assay validation and contains sufficient materials to run ten western blot analyses for each marker under specified conditions.

1 ea

- **Measure autophagy using established markers, LC3-II and p62**
- **Use high-purity Bafilomycin A_1 to assess autophagic flux**
- **Determine effect of your experimental conditions on autophagy**
- **Identify and characterize autophagy activators or inhibitors**

Special offer. See page 11

Autophagy/Cytotoxicity Dual Staining Kit 600140

Summary: Cayman's Autophagy/Cytotoxicity Dual Staining Kit provides a convenient tool for studying the regulation of autophagy and cytotoxicity at the cellular level. The kit employs MDC, an autofluorescent substance incorporated into multilamellar bodies by both an ion trapping mechanism and the interaction with membrane lipids, as a probe for detection of autophagic vacuoles in cultured cells. Propidium iodide is used as a marker of cell death.

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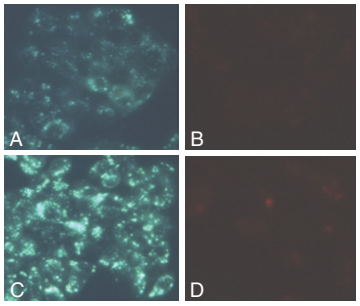

Tamoxifen increases autophagy but not cell death in HepG2 cells as measured by fluorescence microscopy. *Panel A:* MDC staining of HepG2 cells treated with vehicle. There is a basal level of autophagy, indicated by faint silver dot staining of autophagic vacuoles. *Panel B:* Propidium iodide staining of HepG2 cells treated with vehicle. There are few dead cells with only background staining of propidium iodide. *Panel C:* MDC staining of HepG2 cells treated with 10 µM tamoxifen. Note the increase in fluorescence intensity and numbers of autophagic vacuoles compared to the cells treated with vehicle. *Panel D:* Propidium iodide staining of HepG2 cells treated with 10 µM tamoxifen, showing a similar staining pattern to that of cells treated with vehicle.

Which Autophagy Detection kit is right for you?

Special offer. See page 11

Item No.	Product Name	Autophagy Detection Probe(s)	Additional Key Reagent	Detection Method(s)	Notes
15980	Autophlux Kit	LC3B detection antibody p62 detection antibody	Bafilomycin A_1 (inhibitor of vacuolar ATPases)	Western blot	The LC3B antibody detects both LC3B-I and LC3-II. It is isoform specific and does not cross react with LC3A, LC3C, or GABARAP proteins. The p62 antibody binds both wild-type and mutated forms of p62. It reacts with p62 proteins from human, rat, and mouse.
600140	Autophagy/ Cytotoxity Dual Staining Kit	MDC, a nonspecific autophagic vacuole tracer (see notes)	Propidium Iodide (marker of cell death)	Fluorescence microscopy or plate reader	MDC is a nonspecific marker that labels all acidic compartments: endosomes, lysosomes, and autophagic vacuoles. Staining is only obtained when the target compartments are acidic (caution: autophagosomes are not generally acidic) and its accumulation depends on an effective interaction of MDC with autophagic vacuole membrane lipids.

Detect LC3/GABARAP binding proteins

The light chain 3 (LC3) proteins are ubiquitin-like proteins that have dynamic roles in autophagosome formation and processing. While some interact with microtubules, additional partners remain to be identified. This kit provides a simple, powerful approach to identifying proteins that interact with several LC3 family members.

LC3 Interact Kit 15977

Summary: Two families of ubiquitin-like proteins, the LC3 and GABARAP families, are involved in autophagosome formation and function. Cayman's LC3 Interact Kit facilitates the selective capture of LC3- and GABARAP-binding proteins from lysates. The kit comprises of LC3A, LC3B, LC3C, GABARAP, GABARAPL1, and GABARAPL2 agarose conjugates together with an simple spin purification system for efficient protein isolation. Each kit contains sufficient supply of each LC3/GABARAP agarose conjugate to perform five binding assays (30 in total).

1 ea

- **Capture LC3- and GABARAP-binding proteins from biological samples**
- **Determine LC3/GABARAP selectivity of specific interacting proteins *in vitro***
- **Identify LC3- and GABARAP-binding proteins by western blotting or proteomic analysis**
- **Investigate the role of specific LC3 isoforms in different autophagy processes**

Acridine Orange 14338

[494-38-2] C.I. 46005B, NSC 194350
MF: $C_{17}H_{19}N_3$ **FW:** 265.4
A crystalline solid
Summary: A cell-permeable, fluorescent dye that accumulates in acidic organelles in a pH-dependent manner and is used for autophagy detection and cell cycle determination; emits green fluorescence (ex. max: 502 nm; em. max: 525 nm) at neutral pH or when bound to dsDNA and red fluorescence (ex. max: 460 nm; em. max: 650 nm) at acidic pH or when bound to ssDNA or RNA

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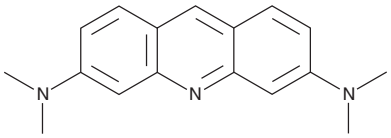

ON OUR COVER
The Harlequin Shrimp

This saltwater shrimp received its name due to its vividly patterned exterior resembling harlequin costumes. Generally found in mating pairs, female shrimp are often larger than their male counterparts. The shrimp's brightly colored exterior and large claws deter predation, which is key to survival, as the shrimp move at a slow pace.

Q&A PAUL DOMANSKI

Antibody Manager, Cayman Chemical Company

Why do you find autophagy interesting and exciting?

Autophagy occurs at a basal level in all cells and was mainly thought of as a non-selective process. A selective side to autophagy is now known, and while some of the proteins involved in the recognition step have been identified, much more work needs to be done. I am intrigued by the role of autophagy in various disease states. Since many neurodegenerative diseases are characterized by the accumulation of misfolded proteins, and these cells cannot divide, autophagy may play a critical role in the pathology of Alzheimer's and similar diseases. Secondly, I am interested in the link between aging and autophagy, as studies suggest that an increase in autophagy might add to longevity.

Why do you study autophagy?

The specific regulatory pathways in each tissue need to be elucidated in order for there to be a concerted effort to produce selective therapies to modulate autophagy. My work at Cayman involves developing unique reagents that can be used by investigators to aid in this process. Our satisfaction is in knowing that something we produced can be instrumental in furthering the general understanding of processes such as autophagy and ubiquitination.

INDUCING AUTOPHAGY

Various signaling pathways converge to regulate autophagy with an initial step of induction that involves membrane nucleation, controlled by the ATG/ULK complex and Beclin 1. mTOR is a major negative regulator of autophagy induction and is thought to directly target ULK. Inhibition of NAMPT, the rate-limiting enzyme involved in NAD^+ synthesis, also leads to autophagy.

mTOR Pathway Inhibitors

Item No.	Product Name	Summary
14745	Cysmethynil	An indole-based, time-dependent inhibitor of Icmt (K_i = 0.14 µM for the final complex); decreases mTOR signaling, accumulation of cells in the G_1 phase, and autophagy-mediated cell death in PC3 prostate cancer cells
11012	Delphinidin (chloride)	A natural plant pigment that suppresses the expression of ERα, inducing both apoptosis and autophagy at concentrations of 1-40 µM
11967	Dorsomorphin	Reversible AMPK inhibitor (K_i = 109 nM) that downregulates the Akt/mTOR pathway to induce autophagy in U251 human glioma cells
13597	Ku-0063794	Selective dual inhibitor of mTORC1 and mTORC2 (IC_{50} = 10 nM)
16982	Perhexiline (maleate)	A CPT1 and CPT2 inhibitor that inhibits rat heart and liver CPT1 (IC_{50}s = 77 and 148 µM, respectively), and rat heart CPT2 (IC_{50} = 79 µM); inhibits mTORC1 signaling and induces autophagy in MCF-7 cells at low µM levels
12006	Rottlerin	Inhibits PKCδ (IC_{50} = 3 µM), CAM kinase III, and a wide range of protein kinases, including PRAK and MAPKAP-K2 (IC_{50}s = 1.9 and 5 µM, respectively); stimulates autophagy by targeting a signaling cascade upstream of mTORC1
13258	Tamoxifen	A SERM that acts as an ER antagonist in breast tissue; stimulates autophagy by increasing the intracellular level of ceramide, which inhibits mTOR activation
10997	Torin 1	Selective mTORC1 and mTORC2 inhibitor (IC_{50}s = 2 and 10 nM, respectively)
14185	Torin 2	Selective mTOR inhibitor (EC_{50} = 0.3 nM) with more than 800-fold selectivity for mTOR over PI3K (EC_{50} = 200 nM) and improved bioavailability compared to Torin 1

*** Also Available: Tamoxifen (citrate) Item No. 11629**

Many more mTOR pathway inhibitors can be found at www.caymanchem.com

Additional Autophagy Inducers

Item No.	Product Name	Summary
11505	ABT-888 (hydrochloride)	An orally bioavailable inhibitor of PARP1 and PARP2 (K_is = 5.2 and 2.9 nM, respectively); enhances apoptosis and autophagy in response to treatments that cause DNA breaks, including radiation and DNA alkylation
13670	CAY10618	A potent inhibitor of NAMPT (IC_{50} = 3 nM); induces cell death in the neuroblastoma cell line SH-SY5Y with an IC_{50} value of 3.8 nM through a process that appears to involve autophagy
13287	FK-866	A highly specific non-competitive inhibitor of NAMPT (K_i = 0.4 nM), causing gradual NAD^+ depletion
14325	2-deoxy-D-Glucose	A non-metabolizable glucose analog used as a glycolytic inhibitor; causes cell cycle inhibition and cell death in *in vitro* models of hypoxia, induces autophagy, increases ROS production, activates AMPK, and blocks tumor cell growth
11499	Obatoclax (mesylate)	An antagonist of Bcl-2, Bcl-W, Bcl-XL, and Mcl-1 (K_D = ~500); prevents interaction of these pro-survival proteins with Bax or Bak, inducing apoptosis and autophagy
16092	Oenin	A natural anthocyanin that stimulates autophagy in U2OS cells
13084	STF-62247	Induces autophagy and selectively causes lethality in renal cell carcinoma cells that have lost von Hippel-Lindau tumor suppressor activity (IC_{50} = 625 nM)
15374	Taurolidine	Binds to the extracellular wall of bacteria, blocking adherence to epithelial and fibroblast cells; induces autophagy, apoptosis, and necrosis in human cancer cells

BLOCKING AUTOPHAGY

Autophagy can be blocked by inhibitors of positive regulators of the ATG/ULK complex and Beclin 1. These include inhibitors of MAP kinases, JNK1, ERK, and p38. Inhibitors of the class III PI3 kinases also block autophagy. In later stages of the autophagic process, inhibitors of lysosome acidification can prevent the formation of autophagosomes and autophagic degradation.

ATPase & Autophagosome Inhibitors

Item No.	Product Name	Summary
11038	Bafilomycin A_1	A selective, reversible inhibitor of V-ATPases (IC_{50}s = 4-400 nM); inhibits autophagy by preventing vacuolar acidification necessary for autophagosome maturation
14005	Bafilomycin B_1	A selective, reversible inhibitor of V-ATPases (IC_{50}s = 4-400 nM)
15318	DBeQ	A selective, reversible, and ATP-competitive inhibitor of the ATPase p97 (K_i = 3.2 µM; IC_{50} = 1.5 µM); blocks ER-associated degradation, impairing the autophagy pathway and promoting the activation of caspase-3 and -7 in cancer cells
13242	3-Methyladenine	Inhibits class I, II, and III PI3Ks, including some downstream targets; at 5 mM, inhibits protein degradation in rat hepatocytes by 65%
17373	ML-240	An ATP-competitive inhibitor of the D2 domain of the p97 ATPase (IC_{50} = 0.11 µM; K_i = 0.22 µM); disrupts ERAD and autophagy pathways and blocks proliferation of cancer cells, rapidly mobilizing caspase-3 and -7 to induce apoptosis
13326	Pifithrin-α	An inactivator of p53 that blocks p53-dependent transcriptional activation, autophagy, and apoptosis
10748	Pifithrin-µ	Inhibits p53 binding to mitochondria; interacts selectively with HSP70; inhibits later stages of the autophagic pathway
10010466	SP 600125	A reversible inhibitor of JNK1-3 (IC_{50} = 0.11 µM); inhibition of JNK activity has been associated with downregulation of Beclin 1 and reduced autophagy
70970	U-0126	Selective MEK1 and MEK2 inhibitor (IC_{50}s = 72 and 58 nM, respectively), and thus is an ERK activator used to study the role of ERK, a MAPK involved in the induction of autophagy

*** Also Available: Cyclic Pifithrin-α (hydrobromide) Item No. 14748**
***p*-nitro-Pifithrin-α Item No. 16209**

Additional PI3K, p38, and MAPK inhibitors can be found at www.caymanchem.com

Antibodies

Item No.	Product Name	Antigen	Reactivity	Host	Application(s)
11512	HMGB1 Monoclonal Antibody (Clone IMG19N10B7)	Full length recombinant human HMGB1	(+) Human and mouse HMGB1	Mouse	FC, IHC (paraffin-embedded tissue), WB
11513	HMGB1 Monoclonal Antibody (Clone IMG19N15F4)	Full length recombinant human HMGB1	(+) Human and mouse HMGB1	Mouse	FC, IHC (paraffin-embedded tissue), WB
11515	HMGB1 Polyclonal Antibody (aa 100-150)	Human HMGB1 amino acids 100-150	(+) Human, bovine, chicken, mouse, New World monkey, and rat HMGB1	Rabbit	FC, IHC (paraffin-embedded tissue), WB
10011444	LAMP2 Monoclonal Antibody (Clone GL2A7)	Purified preparation of mouse liver lysosomal membranes	(+) Mouse and rabbit LAMP2	Rat	ICC, IP

ask Cayman QUESTIONS FROM THE FIELD

Q: What is on the horizon for autophagy research?

A: Although autophagy has been known for quite some time, researchers are still in the early stages of fully understanding the processes behind it. Only recently have therapeutic modulators been developed. In the past 10 years, publications surrounding autophagy have increased 10-fold, yet much is to be discovered concerning the actual players in various tissues. Many questions remain, including: How are autophagy and apoptosis related? How does autophagy interact with the ubiquitin-proteasome system? How is autophagy regulated in growth and differentiation? How can the pathway be manipulated to treat various diseases?

PROTEASOME PROTEOLYTIC ACTIVITY

Special offer. See page 11

20S Proteasome Assay Kit 10008041

Summary: The 20S proteasome is the proteolytic core of a large protein degradation complex, the 26S proteasome. Proteasome inhibitors exhibit anti-inflammatory and antiproliferative effects, evidence that the proteasome may be an important drug target for the treatment for cancer and inflammatory diseases. Cayman's 20S Proteasome Assay employs a specific 20S substrate, SUC-LLVY-AMC which, upon cleavage by the active enzyme, generates a highly fluorescent product with an emission wavelength at 480 nm.

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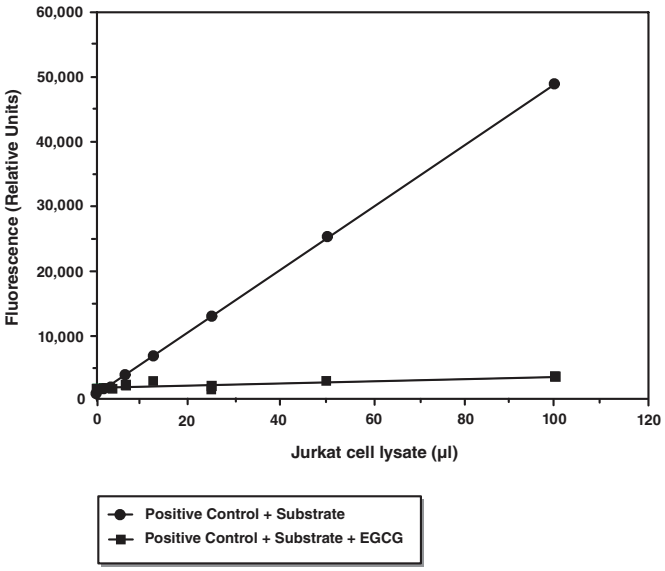


ask Cayman

QUESTIONS FROM THE FIELD

Will the 20S Proteasome Assay Kit work with frozen cells or only on freshly cultured cells?

In general, this kit should be used with fresh cells. However, detection in frozen cells may be possible, depending upon the level of 20S proteasome activity within the sample. For example, the positive control provided in this kit is a Jurkat cell lysate supernatant that contains a very high level of 20S activity and is stored frozen at -80˚C. Many cell types do not have as robust 20S proteasome activity as that of Jurkat cells and, upon freezing, may lose activity to a point below the detection limit.

Proteasome Inhibitors

Item No.	Product Name	Summary
14921	Calpain Inhibitor I	A synthetic tripeptide aldehyde that inhibits cysteine proteases, including calpain I (K_i = 190 nM), calpain II (K_i = 220 nM), cathepsin B (K_i = 150 nM), and cathepsin L (K_i = 500 pM)
15994	Calpain Inhibitor II	A cell permeable, peptide aldehyde inhibitor of calpain I (K_i = 120 nM), calpain II (K_i = 230 nM), cathepsin B (K_i = 100 nM), and cathepsin L (K_i = 0.6 nM)
15303	Disulfiram	A copper and zinc chelator and an irreversible inhibitor of aldehyde dehydrogenase (IC_{50} = 0.1 mM) that targets the ubiquitin-proteasome pathway, inhibiting purified 20S proteasome (IC_{50} = 7.5 µM) and 26S proteasome (IC_{50} = 20 µM)
10007713	HMB-Val-Ser-Leu-VE	A selective, cell-permeable inhibitor of the trypsin-like activity of the 20S proteasome (IC_{50} = 0.33 µM)
70980	Lactacystin	A microbial metabolite isolated from *Streptomyces* that is widely used as a selective inhibitor of the 20S proteasome
70988	Clasto-Lactacystin β-lactone	Active metabolite of lactacystin, with at least 10 times better activity; irreversibly alkylates subunit X of the 20S proteasome
15413	(S)-MG115	A reversible proteasome inhibitor, targeting the chymotryptic site on the 20S particle (K_i = 21 nM)
13697	(R)-MG132	A more effective inhibitor of chymotrypsin-like, trypsin-like, and peptidylglutamyl peptide hydrolyzing proteasome activities compared to (S)-MG132 (IC_{50}s = 0.22 *versus* 0.89 µM (ChTL); 34.4 *versus* 104.43 µM (TL); 2.95 *versus* 5.70 µM (PGPH), respectively)
10012628	(S)-MG132	A reversible, cell permeable proteasome inhibitor
16269	ONX 0912	An orally bioavailable inhibitor of CT-L activity of 20S proteasome β5 and LMP7 (IC_{50}s = 36 and 82 nM, respectively)
16271	ONX 0914	A selective inhibitor of immunoproteasomes with minimal cross reactivity for the constitutive proteasome

UBIQUITINATION

Ubiquitination is an enzymatic PTM that labels specific proteins for degradation by the 26S proteasome. A multi-enzyme cascade involving ubiquitin-activating enzymes (E1s), ubiquitin-conjugating enzymes (E2s or UBCs), and ubiquitin-protein ligases (E3s) mediates the ubiquitination process. Ubiquitination plays a role in numerous cellular functions, including apoptosis, signaling, cell cycling and division, DNA transcription, and immune responses.

Ubiquitinated Protein Detection

Special offer. See page 11

Ubiquitin Interact Kit 15978

Summary: Cayman's Ubiquitin Interact Kit facilitates the selective capture and detection of ubiquitin binding and associated proteins from cell lysates and tissue extracts. The kit utilizes a high capacity, high specificity ubiquitin matrix together with an easy-to-use spin purification system for efficient isolation of ubiquitin binding proteins with minimal nonspecific binding. Each kit contains sufficient Ubiquitin Interact Matrix to perform up to 20 binding assays.

1 ea

Special offer. See page 11

Ubiquitin Link Kit 15985

Summary: Cayman's Ubiquitin Link Kit provides a flexible, easy-to-use system for developing and running ubiquitination assays *in vitro*. The kit contains a range of E2 conjugating enzymes together with high-grade ubiquitin for enhanced E3 auto-ubiquitination and substrate ubiquitination. Assays can be analyzed by western blotting, proteomic, and biochemical methods. Each kit contains sufficient ubiquitin-conjugating enzymes for four reactions per E2 supplied (up to 50 reactions in total).

1 ea

Special offer. See page 11

Ubiquitinated Protein Capture Kit 15979

Summary: Cayman's Ubiquitinated Protein Capture Kit facilitates the fast, effective capture and detection of ubiquitinated proteins from biological samples. The kit utilizes a high capacity, high specificity ubiquitin binding matrix together with an easy-to-use spin purification system for less 'hands-on time' and superior performance. This purification system is highly adaptable and compatible with samples from various species and with a broad range of lysis buffers. Each kit contains sufficient Ubiquitin Matrix to perform up to 20 assays.

1 ea

Inhibitors of Ubiquitin-Activating and -Conjugating Enzymes

Item No.	Product Name	Summary
16259	JNJ-26854165	An MDM2 antagonist that suppresses the growth of cancer cell lines expressing wild-type p53 (IC_{50}s = 240-440 nM)
15217	MLN 4924	Selectively inhibits the NEDD8-activating enzyme (IC_{50} = 4.7 nM); also inhibits ubiquitin-activating enzyme and SUMO-activating enzyme (IC_{50}s = 1.5 and 8.2 µM, respectively)
15226	PYR41	A cell-permeable, irreversible E1 inhibitor (IC_{50} < 10 µM); blocks ubiquitination and prevents ubiquitin-mediated proteasomal degradation; causes an increase in sumoylation of proteins
15324	SMER3	A selective inhibitor of SCF^{MET30} ubiquitin ligase, an E3 ligase that regulates transcription, cell-cycle control, and immune response
15964	TZ9	A cell-permeable inhibitor of the human E2 ubiquitin-conjugating enzyme Rad6B

Antibodies

Item No.	Product Name	Antigen	Reactivity	Host	Application(s)
14219	Multiubiquitin Chain Monoclonal Antibody (Clone FK1)	Crude preparation of polyubiquitinated-lysozyme	(+) Polyubiquitinated conjugates (-) Free ubiquitin and monoubiquitinated conjugates	Mouse	ELISA, IHC, WB
14220	Multiubiquitin Chain Monoclonal Antibody (Clone FK2)	Crude preparation of polyubiquitinated-lysozyme	(+) Polyubiquitinated and monoubiquitinated conjugates (-) Free ubiquitin	Mouse	ELISA, IHC, WB
13722	Ubiquitin Monoclonal Antibody (Clone 5B9-B3)	Native bovine ubiquitin conjugated to KLH	(+) Human, bovine, mouse, and rat ubiquitin	Mouse	ELISA, WB
13723	Ubiquitin Monoclonal Antibody (Clone 6C11-B3)	Native bovine ubiquitin conjugated to KLH	(+) Human, bovine, mouse, and rat ubiquitin	Mouse	ELISA, WB
13724	Ubiquitin Polyclonal Antibody	Native bovine ubiquitin conjugated to KLH	(+) Bovine, chicken, canine, *Drosophila*, guinea pig, hamster, human, monkey, mouse, ovine, porcine, rainbow trout, rat, *Xenopus*, and yeast ubiquitin	Rabbit	ChIP, IP, WB

DEUBIQUITINATING ENZYME ACTIVITY

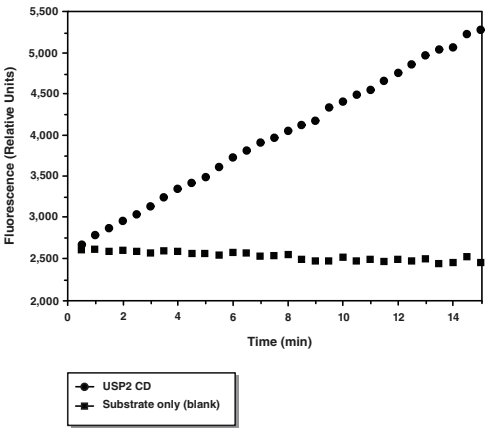
Special offer. See page 11

DUB Activity Assay Kit 15981

Deubiquitinating Enzyme

Summary: DUBs remove ubiquitin from modified proteins in order to recycle ubiquitin attached to inappropriate targets, remove and disassemble polyubiquitin chains, and process proteins prior to their degradation by the proteasome. They have been implicated in a number of human diseases and, thus, are attractive targets for potential therapeutic intervention *via* the development of suitable inhibitors and modulators. Cayman's DUB Activity Assay Kit facilitates the rapid, robust measurement of deubiquitinating enzyme activity *in vitro*. The kit utilizes a high purity, fluorogenic substrate (ubiquitin-AMC) together with suitable calibration standards and controls for the accurate and sensitive assessment of DUB activity. Continuous kinetic or end-point assays can be performed in a 96-well plate format for multi-sample analysis.

96 wells



Deubiquitinating Enzyme Inhibitors

Item No.	Product Name	Summary
11324	b-AP15	Inhibits USP14 and UCHL5, two proteasome-associated deubiquitinases; inhibits DUB activity in purified 19S proteasomes (IC_{50} = 2.1 µM)
10617	IU1	A reversible, small molecule inhibitor of USP14 deubiquitination (IC_{50} = 4-5 µM); stimulates ubiquitin-dependent protein degradation *in vitro* (34 µM) and in cells (50 µM)
16893	NSC 632839	An inhibitor of the DUBs USP2 and USP7 as well as the ubiquitin-like SUMO peptidase SENP2 (EC_{50}s = 45, 37, and 9.8 µM, respectively)
15224	P005091	Selectively inhibits USP7 and the closely related USP47 (EC_{50}s = 4.2 and 4.3 µM, respectively); accelerates the degradation of the USP7 substrate HDM2 in several multiple myeloma cell lines (EC_{50} = 11 µM)
16353	TCID	Inhibits UCH-L3 and demonstrates 125-fold selectivity over UCH-L1 (IC_{50}s = 0.6 and 75 µM, respectively)
15227	WP1130	Inhibits the deubiquitinase activity of USP9x, USP5, USP14, and UCH37; 5 µM induces the accumulation of protein-ubiquitin conjugates, resulting in the formation of aggresomes and apoptosis in a variety of tumor cells

WHICH UBIQUITIN KIT IS RIGHT FOR YOU?

Special offer. See page 11

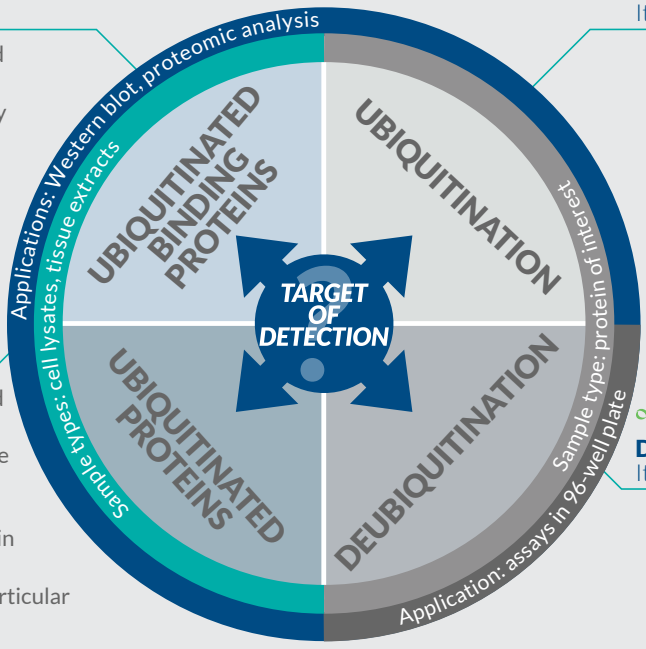
Ubiquitin Interact Kit
Item No. 15978

- Identify and characterize isolated ubiquitin binding proteins
- Demonstrate ubiquitin binding by specific proteins of interest
- Investigate role of ubiquitin in particular signaling pathways

Special offer. See page 11

Ubiquitinated Protein Capture Kit
Item No. 15979

- Capture and detect ubiquitinated proteins and free chains
- Demonstrate specific proteins are substrates for ubiquitin modification *in vivo*
- Identify and characterize ubiquitin modified proteins
- Investigate role of ubiquitin in particular signaling pathways



Special offer. See page 11

Ubiquitin Link Kit
Item No. 15985

- Develop assays for E3 auto-ubiquitination and substrate ubiquitination
- Determine suitable E2 for use with specific E3 or E3-substrate systems
- Assess E3 ligase activity of novel enzymes
- Investigate effect of ubiquitination on substrate protein function

Special offer. See page 11

DUB Activity Assay Kit
Item No. 15981

- Assess performance of specific DUBs
- Evaluate performance of DUB activators and inhibitors
- Screen for potential activators and inhibitors

European Platform

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