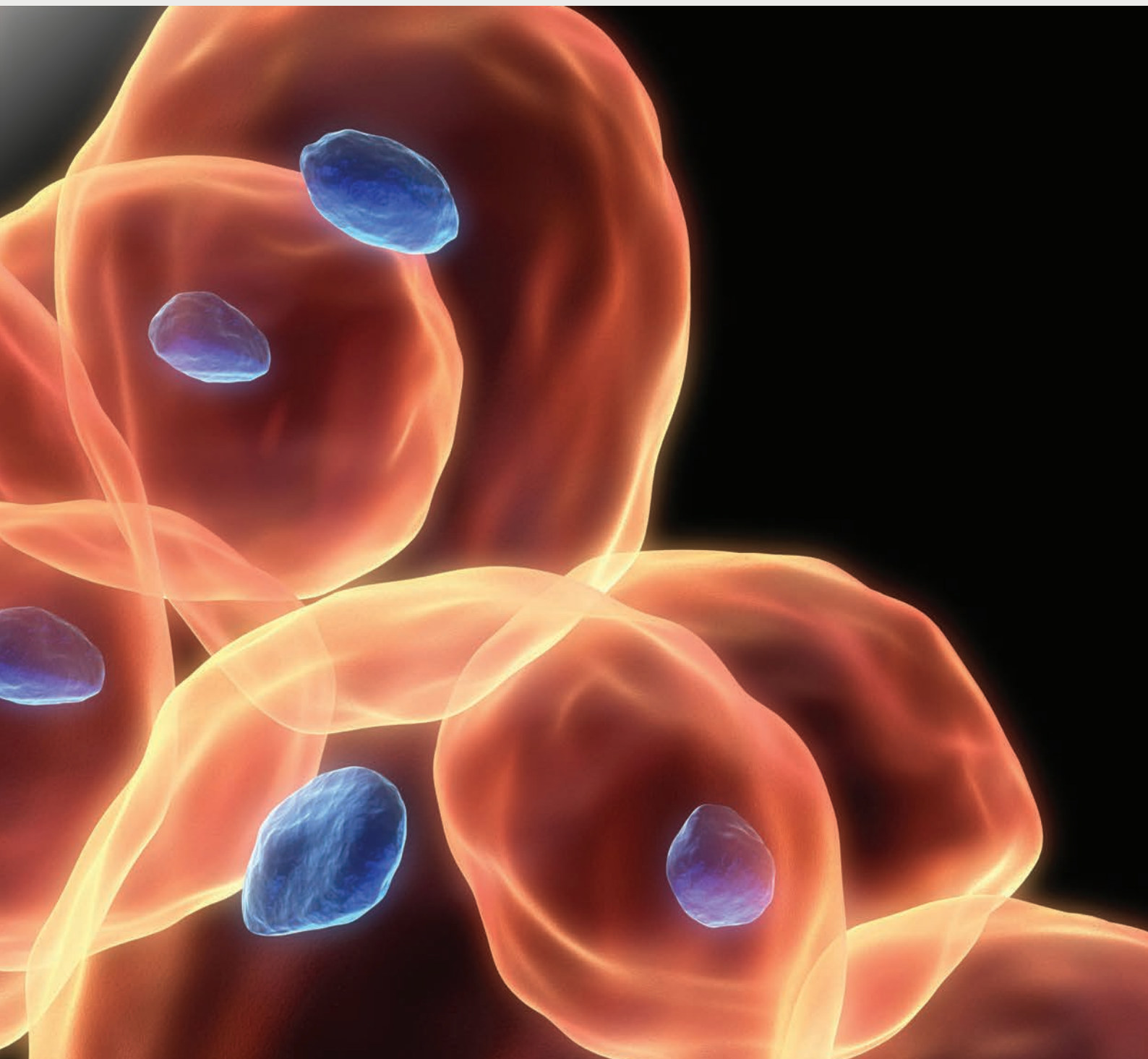




# Autophagy

Innovative Tools for Analysis



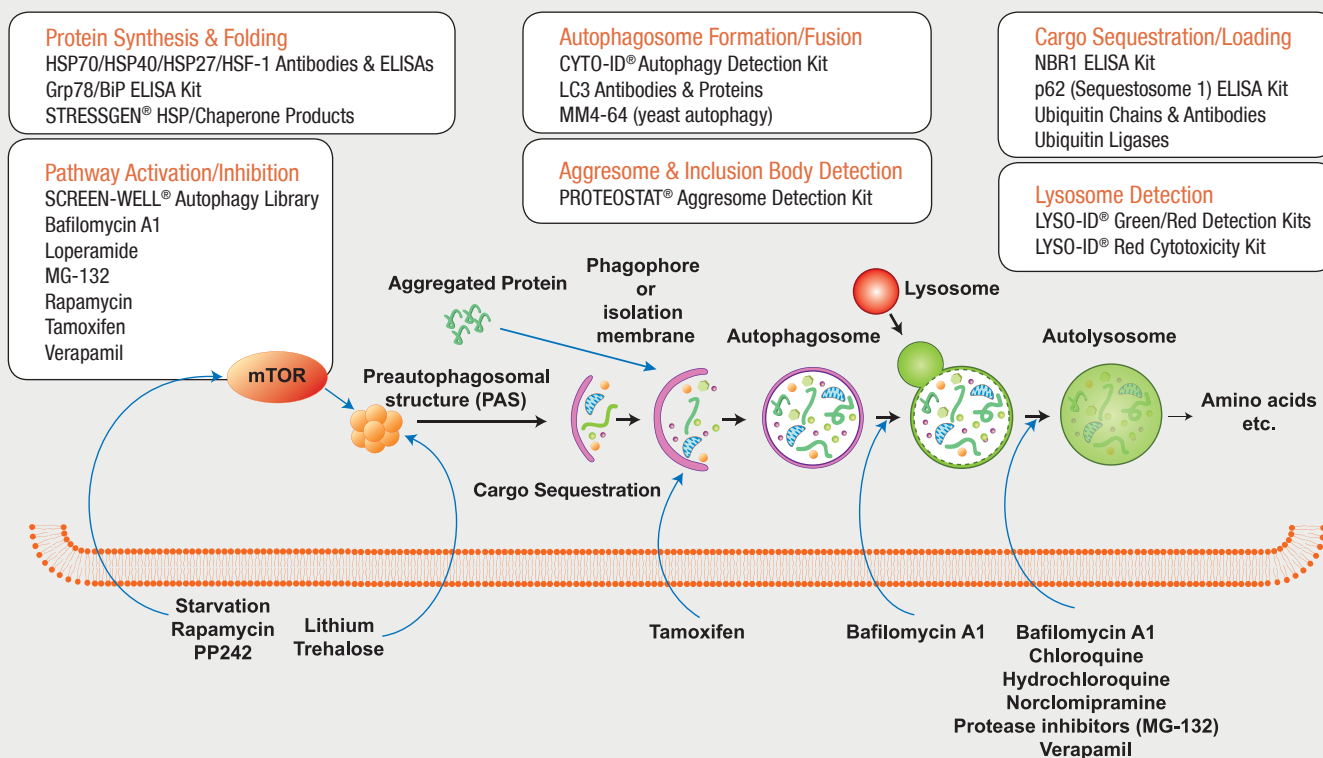
The image features a background of orange-toned microscopic cell structures, possibly showing organelles like nuclei and membranes. A white arrow points to the left, positioned to the left of the text.

FIND YOUR AUTOPHAGY SOLUTION

# COMPREHENSIVE SOLUTIONS FOR AUTOPHAGY

## Enabling a Clearer Understanding of Autophagy and Disease Through Innovative Assays

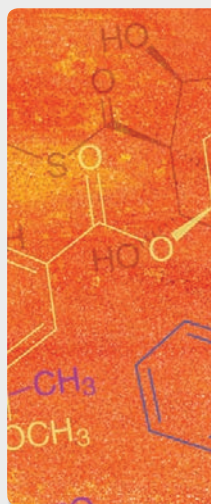
Autophagy (literally “self-eating”) is a lysosome-mediated intracellular bulk degradation pathway employed by eukaryotic cells when subjected to certain hostile conditions (such as nutrient deprivation) that triggers digestion and recycling of cellular contents. Various cytoplasmic constituents, including organelles, aggregated proteins, and long-lived proteins are sequestered into double-membrane autophagosomes, which subsequently fuse with lysosomes where their contents are degraded. Autophagic activity is critical to the maintenance of cellular homeostasis and energy balance. Although typically low under basal conditions, autophagy can be markedly upregulated by a variety of physiological stimuli such as nutrient starvation, hypoxia, endoplasmic reticulum stress, as well as immune and hormonal stimulation. Mounting evidence has connected malfunctions in autophagic processes to many clinically relevant diseases including cancer, neurodegeneration, diabetes, autoimmunity, and cardiovascular disease. Development of autophagy-targeting therapies will depend on a deeper understanding of the benefits, and potential consequences, of altering autophagic activity.



### Cell-Based Assays & Biomarker Detection

Highlighted by the innovative CYTO-ID® Autophagy Detection Kit, Enzo's panel of CELLESTIAL® assays includes unique fluorescent-probe based assays for detection of lysosomes, aggresomes, and apoptosis/necrosis.

Our portfolio of over 300 ELISA & detection kits includes sensitive, specific assays for relevant markers of autophagy and autophagy-regulating pathways. Over 40 years of assay development experience and state-of-the-art manufacturing facilities ensure time-tested reproducibility.



### Small Molecule Chemistry & Compound Libraries

Included in our thousands of stand-alone small molecules is a variety of biologically characterized autophagy activators and inhibitors. We've assembled them into a convenient SCREEN-WELL® Autophagy Library to facilitate high content screening of novel compounds against those with defined autophagy-modulating properties.

Through our supplier network built over a 40 year history, we have the ability to rapidly and inexpensively source traditional, custom, and bulk compounds in the gram to kilogram scale. We complete our sourcing using stringent quality control standards with state-of-the-art methods.

# MEASURE BIOMARKERS P62 & NBR1 THROUGH ELISA ANALYSIS

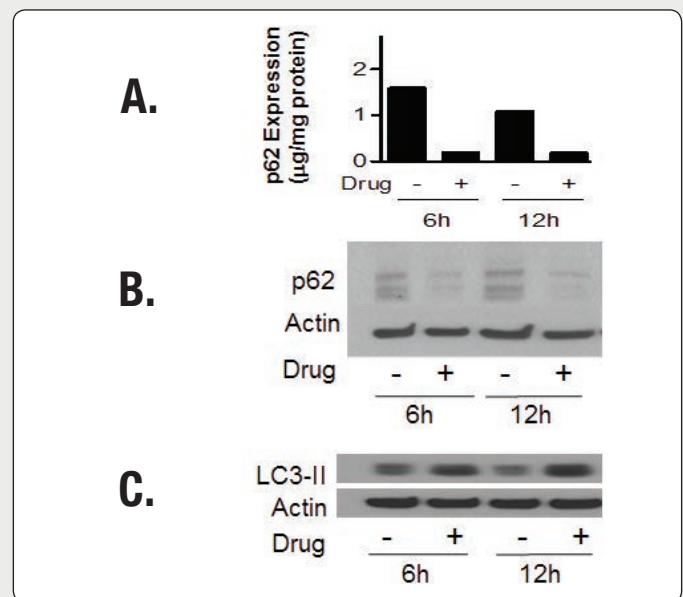
Sensitive NBR1 and p62 ELISA Kits allow for quantitative, immunometric detection of the autophagy biomarkers NBR1 and p62 (Sequestosome 1) in human, rat and mouse cell lysates. NBR1 and p62 function as scaffold proteins, aiding in autophagy protein trafficking and degradation. ELISA kits enable quantitative measurement of autophagy without the need for expensive equipment or long procedures.

- Sensitive assays measure as little as 66 and 100 pg/ml of NBR1 and p62, respectively
- Fully quantitative results surpass semi-quantitative Western blot analysis
- High throughput format allows analysis of up to 40 samples in duplicate in less than 3 hours
- Easy-to-follow protocols and liquid color-coded reagents save time and reduce errors

PRODUCT	PRODUCT #	SIZE
NBR1 ELISA Kit	ADI-900-211	1 x 96 wells
p62 ELISA Kit	ADI-900-212	1 x 96 wells

SELECT RELATED ELISA KITS	
Product Name	Product #
Bax	ADI-900-138
Bcl-2	ADI-900-133
Erk1/2	ADI-900-152
Grp78/BiP	ADI-900-214
GSK-3 $\beta$	ADI-900-144
HSP70	ADI-EKS-715
HSP90 $\alpha$	ADI-EKS-895
p53	ALX-850-057
p53/MDM2	ADI-960-070
Phospho GSK-3 $\beta$	ADI-900-123A
Phospho Jnk1/2	ADI-900-106

## p62 ELISA Measures Induction of Macroautophagy



MDA-MB-231 human breast cancer cells were treated with 2 $\mu$ M of withaferin A (WA), an autophagy inducing drug or vehicle. Cells were harvested 6 and 12 hours post-treatment, lysed, and analyzed with the p62 ELISA Kit, and for p62 and LC3-II by Western blot. Drug treatment correlated with the induction of autophagy as indicated by the decrease in p62 levels (A and B) and by elevation of LC3-II levels (C).

## Sensitive Autophagy Antibodies & Pure Proteins

We offer a large selection of purified proteins and highly validated monoclonal and polyclonal antibodies against peptides, whole or partial proteins, and modified peptides or proteins. Many of our antibodies are also available as conjugates to proteins or fluorescent labels, expanding their utility in a variety of common applications including Western blot, flow cytometry, IHC, ICC, IF, IP, and more.

SELECT RELATED ANTIBODIES AND PROTEINS			
Product Name	Product #	Product Name	Product #
Beclin-1, pAb	ADI-905-721	LC3B, mAb (5F10)	ALX-803-080
HSC70/HSP70, mAb (N27F3-4)	ADI-SPA-820	Mono & Poly Ub conjugates, mAb (FK2)	BML-PW8810
HSP27 (human), (recombinant)	ADI-SPP-715	mTOR (human FRB Domain), pAb	ALX-215-065
HSP70 (low endotoxin) (human), (recombinant)	ADI-ESP-555	mTOR, pAb	ADI-905-687
HSP70/HSP72 (human), (recombinant)	ADI-NSP-555	NBR1 (human), pAb	BML-PW1125
HSP90 $\alpha$ (human), (recombinant)	ADI-SPP-776	p62 (human), pAb	BML-PW9860

Enzo has over 35 Heat Shock Proteins. Learn more at [www.enzolifesciences.com/HSP](http://www.enzolifesciences.com/HSP)



# QUANTIFY AUTOPHAGY IN LIVE CELLS WITHOUT TRANSFECTION

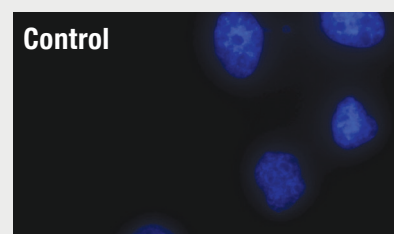
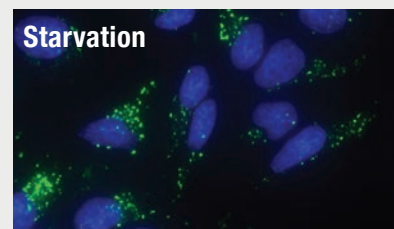
The CYTO-ID® Autophagy Detection Kit 2.0 (ENZ-KIT175) is a no-transfection quantitative assay for monitoring autophagy in live cells. Suitable for flow cytometry, microscopy, and micorplate assays. The assay facilitates high-throughput screening of activators and inhibitors of autophagy. The CYTO-ID Autophagy Detection Kit 2.0 includes a patented dye that selectively stains pre-autophagosomes, autophagosomes, and autolysosomes (autophagolysosomes) with minimal staining of lysosomes.

PRODUCT	PRODUCT #	SIZE (50 OR 200 TESTS)
CYTO-ID® Autophagy Detection Kit 2.0	ENZ-KIT175	50 flow cytometry assays, 60 microscopy assays or 1 x 96-well microplate assays; 200 flow cytometry assays, 250 microscopy assays or 3 x 96-well microplate assays

- Proprietary dye selectively stains autophagic vesicles, eliminating the need for time-consuming LC3-fusion protein transfection
- Rapidly quantifies autophagy in native heterogeneous cell populations
- Validated under a wide range of conditions and with small molecule modulators known to influence autophagy pathways
- Negligible staining of lysosomes reduces background seen with other dyes (e.g. MDC)

SELECT RELATED LIVE CELL ANALYSIS KITS	
Product Name	Product #
LYSO-ID® Green Detection Kit	ENZ-51034
LYSO-ID® Red Detection Kit (GFP-CERTIFIED®)	ENZ-51005
LYSO-ID® Red Cytotoxicity Kit (GFP-CERTIFIED®)	ENZ-51015
PROTEOSTAT® Aggresome Detection Kit	ENZ-51035
MM4-64 Fluorescent Dye for Autophagy in Yeast	ENZ-52252

## Profile Autophagy Without Transfection



Starvation induces an increase in green fluorescence intensity as demonstrated by the presence of punctate cytoplasmic structures (top) compared to control cells (bottom). Nuclei counterstained with Hoechst dye (blue).

## Screen Autophagy Modulators With Small Molecule Activators & Inhibitors

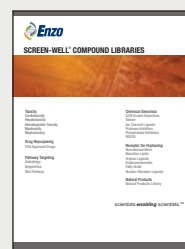
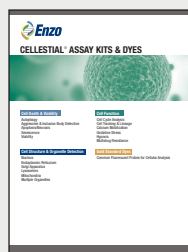
The SCREEN-WELL® Autophagy Library is a useful tool for studying the role of pro- and anti-autophagic molecules using *in vitro* screening assays. This unique collection contains 94 compounds with defined autophagy-inducing or -inhibitory activity, selected from structurally and mechanistically different compound classes known to modulate autophagy-related targets and processes including:

- mTOR/PI3K
- Heat Shock Proteins
- Epigenetic Regulators
- ER Stress
- Calcium Channels
- Proteasome
- Cytoskeleton
- Lysosomal pH
- cAMP
- and more...

PRODUCT	COMPOUNDS	PRODUCT #	SIZE
SCREEN-WELL® Autophagy Library	94	BML-2837	100 µL/well, 500 µL/well

SELECT RELATED INDIVIDUAL COMPOUNDS			
Product Name	Product #	Target	Effect
Bafilomycin A1	BML-CM110	Inhibits vacuolar-ATPase	Inhibits autophagy
Loperamide	ALX-550-253	Ca <sup>2+</sup> channel blocker; reduces intra-cytosolic Ca <sup>2+</sup> levels; mTOR-independent	Activates autophagy
MG-132	BML-PI102	Selective proteasome inhibitor	Activates autophagy
Rapamycin	BML-A275	Inhibits mammalian target of rapamycin (mTOR)	Activates autophagy
SMER28	BML-EI397	mTOR-independent autophagy inducer	Activates autophagy
Tamoxifen	ALX-550-095	Increases the intracellular level of ceramide and abolishes the inhibitory effect of PI3K	Activates autophagy
Verapamil	ALX-550-306	Ca <sup>2+</sup> channel blocker; reduces intracytosolic Ca <sup>2+</sup> levels; mTOR-independent	Activates autophagy

*You may also be interested in:*



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Our broad range of scientific expertise and industry-proven manufacturing capabilities enables us to provide a comprehensive set of solutions for autophagic research.