

Anti-Apg8 (RABBIT) Antibody Apg8 Antibody Catalog # ASR4395

## **Specification**

# Anti-Apg8 (RABBIT) Antibody - Product Information

Host Conjugate Target Species Reactivity Clonality Application Application Note	Rabbit Unconjugated Yeast Yeast Polyclonal WB, IHC, E, IP, I, LCI Anti-Apg8 purified polyclonal antibody reacts with yeast APG8 by western blot and ELISA. This antibody using the specified conditions may recognize other prominent intrinsic bands (UBLs or their conjugates). Other intrinsic bands are readily detectable in yeast lysates at lower antibody dilutions. For immunoblotting a 14 kDa band corresponding to yeast Apg8 is detected. Most yeast cell lysates can be used as a positive control without induction or stimulation. Although not tested, this antibody is likely functional in immunohistochemistry and immunoprecipitation.
Physical State Buffer	Lyophilized 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Immunogen	Rabbit Anti-Apg8 antibody was prepared from rabbit serum after repeated immunizations with recombinant yeast Apg8 protein.
Reconstitution Volume Reconstitution Buffer	100 μL Restore with deionized water (or equivalent)
Preservative	0.01% (w/v) Sodium Azide

# Anti-Apg8 (RABBIT) Antibody - Additional Information

Gene ID 852200

Other Names 852200

Purity

Anti-Apg8 antibody, also known as Atg8, is an IgG fraction antibody purified from monospecific antiserum by a multi-step process which includes delipidation, salt fractionation and ion exchange chromatography followed by extensive dialysis against the buffer stated above. Assay by



immunoelectrophoresis resulted in a single precipitin arc against anti-Rabbit Serum.

### Storage Condition

Store antibody at 4° C prior to restoration. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.

### **Precautions Note**

This product is for research use only and is not intended for therapeutic or diagnostic applications.

## Anti-Apg8 (RABBIT) Antibody - Protein Information

### Name ATG8

### Synonyms APG8, AUT7, CVT5

### Function

Ubiquitin-like modifier involved in cytoplasm to vacuole transport (Cvt) vesicles and autophagosomes formation. With ATG4, mediates the delivery of the vesicles and autophagosomes to the vacuole via the microtubule cytoskeleton. Required for selective autophagic degradation of the nucleus (nucleophagy) as well as for mitophagy which contributes to regulate mitochondrial quantity and quality by eliminating the mitochondria to a basal level to fulfill cellular energy requirements and preventing excess ROS production. Participates also in membrane fusion events that take place in the early secretory pathway. Also involved in endoplasmic reticulum-specific autophagic process and is essential for the survival of cells subjected to severe ER stress. The ATG8-PE conjugate mediates tethering between adjacent membrane during autophagy. Moreover not only conjugation, but also subsequent ATG8-PE deconjugation is an important step required to facilitate multiple events during macroautophagy, and especially for efficient autophagosome biogenesis, the assembly of ATG9-containing tubulovesicular clusters into phagophores/autophagosomes, and for the disassembly of PAS-associated ATG components. Also plays a role in regulation of filamentous growth.

#### **Cellular Location**

Cytoplasmic vesicle, cvt vesicle membrane; Lipid- anchor. Cytoplasmic vesicle, autophagosome membrane; Lipid-anchor. Vacuole membrane; Peripheral membrane protein. Mitochondrion membrane; Peripheral membrane protein. Note=Membrane-associated through a lipid anchor. This association needs the 2 ubiquitin-like systems required for cytoplasm to vacuole transport and autophagy. ATG18 and ATG21 facilitate the recruitment of ATG8-PE to the site of autophagosome formation and protect it from premature cleavage by ATG4. Localizes to both the isolation membrane (IM) and the vacuole-isolation membrane contact site (VICS) during IM expansion. The IM is a membrane sac generated from the pre-autophagosomal structure that ultimately expands to become a mature autophagosome. Upon starvation, is also recruited to into unique membrane structures near SEC13-containing ER exit sites which lack components of the Golgi apparatus and the endosomes, and which were called a compartments for unconventional protein secretion (CUPS). Associates with mitochondria following nitrogen starvation in a respiratory carbon source (PubMed:30510114)

# Anti-Apg8 (RABBIT) Antibody - Protocols

Provided below are standard protocols that you may find useful for product applications.



- <u>Western Blot</u>
- <u>Blocking Peptides</u>
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- <u>Immunoprecipitation</u>
- Flow Cytomety
- <u>Cell Culture</u>

## Anti-Apg8 (RABBIT) Antibody - Images



Western blot of APG8 fusion protein. Anti-APG8 antibody generated by immunization with recombinant yeast APG8 was tested by western blot with other anti-UBL antibodies against E.coli lysates expressing the APG8-GFP fusion protein. All UBLs possess limited homology to Ubiquitin and to each other, therefore it is important to know the degree of reactivity of each antibody against each UBL. Panel A (top) shows total protein staining using ponceau. Panel B (bottom) shows specific reaction with APG8 using a 1:4,000 and 1:8,000 dilution of Rockland's IgG fraction of Rabbit-anti-APG8 (Yeast) followed by reaction with a 1:15,000 dilution of HRP Goat-a-Rabbit IgG MX (code # 611-103-122). All primary antibodies were diluted in TTBS buffer supplemented with 5% non-fat milk and incubated with the membranes overnight at 4° C. E.coli lysate proteins were separated by SDS-PAGE using a 15% gel. Similar experiments (data not shown), where other UBL fusion proteins were separated and probed with this antibody showed no reactivity of anti-APG8 with other UBLs. This data indicates that anti-APG8 is highly specific and does not cross react with other UBLs. A chemiluminescence system was used for signal detection (Roche). Other detection systems will yield similar results. Data contributed by M. Malakhov, www.lifesensors.com, personal communication.

# Anti-Apg8 (RABBIT) Antibody - Background

Anti-Atg8 antibody is an Ubiquitin-like protein (UBL) antibody. UBL proteins and antibodies fall into two classes: the first class, ubiquitin-like modifiers (UBLs) function as modifiers in a manner analogous to that of ubiquitin. Examples of UBLs are SUMO, Rub1 (also called Nedd8), Apg8 and Apg12. Proteins of the second class include parkin, RAD23 and DSK2, are designated ubiquitin-domain proteins (UDPs). These proteins contain domains that are related to ubiquitin but are otherwise unrelated to each other. In contrast to UBLs, UDPs are not conjugated to other proteins. Apg8 is required for autophagy (intracellular bulk protein degradation) in yeast. Starved yeast cells take up their own cytoplasm into vacuoles through autophagic bodies. Autophagic bodies form a double-membraned structure called the autophagosome, which subsequently fuses with the vacuole/lysosome. This process similar in mammals. Two sets of genes, APG and AUT, have been identified with this process, and are responsible for two ubiquitin-like systems Apg12 and Apg8, respectively. Apg12 is synthesized in its mature form and seems to have one target, Apg5. Almost all Apg12 molecules are conjugated with Apg5. Aut2/Apg4 processes the Apg8/Aut7



system at its carboxy-terminal region. Apg8 exists in two forms, one is membrane bound through a phospholipid. Lipidation/activation of Apg8 is mediated by Apg7 and transferred to Apg3 and finally forms a conjugate with phosphatidyl-ethanolamine (PE). Apg4 cleaves Apg8-PE, releasing Apg8 from membrane. Morphological studies show that Apg8 localizes on the membrane of intermediate structures of the autophagosome; this transient association seems to be essential for formation of the autophagosome. Both Apg12 and Apg8 are highly conserved, with apparent homologues in the worm, mammals and plants. In higher eukaryotes, Apg8 consists of a multigene family. Anti-ATG8 Antibody is useful for researcher interested in autophagy, Cell Biology, Microbiology and Signal Transduction research.