

Datasheet for 200-401-439**Apg8 Antibody****Overview**

Description:	Anti-Apg8 (RABBIT) Antibody - 200-401-439
Item No.:	200-401-439
Size:	500 µg
Applications:	WB
Reactivity:	Yeast
Host Species:	Rabbit

Product Details**Background:**

Anti-Apg8 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 is 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 phosphatidylethanolamine (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.

Synonyms:	rabbit anti-APG8 Antibody, APG-8, APG 8, ATG-8, ATG 8, Rabbit Anti-ATG8 Antibody, Autophagy-related protein 8, Autophagy-related ubiquitin-like modifier ATG8, Cytoplasm to vacuole targeting protein 5, AUT7, CVT5, YBL078C, YBL0732
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	ATG8
Reactivity:	Yeast
Immunogen Type:	Recombinant Protein
Immunogen:	Rabbit Anti-Apg8 antibody was prepared from rabbit serum after repeated immunizations with recombinant yeast Apg8 protein.
Purity/Specificity:	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.
Relevant Links:	• UniProtKB - P38182 • NCBI - 6319393 • GeneID - 852200

Application Details

Tested Applications:	WB
Application Note:	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.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:20,000 - 1:100,000
IHC:	User Optimized
IP:	User Optimized

WB: 1:4,000 - 1:8,000

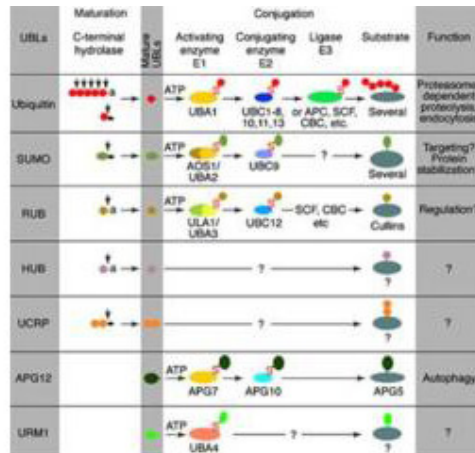
Formulation

Physical State:	Lyophilized
Concentration:	5.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None
Reconstitution Volume:	100 µL
Reconstitution Buffer:	Restore with deionized water (or equivalent)

Shipping & Handling

Shipping Condition:	Ambient
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.
Expiration:	Expiration date is one (1) year from date of receipt.

Images

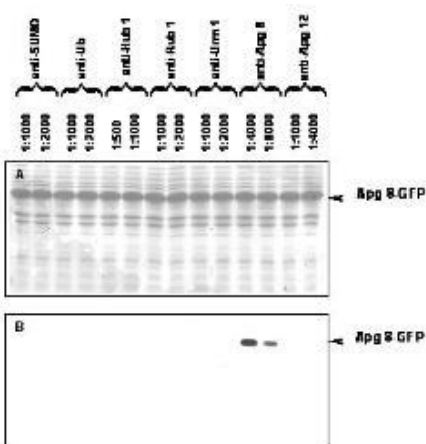


Pathway

Most modifiers mature by proteolytic processing from inactive precursors (a; amino acid). Arrowheads point to the cleavage sites. Ubiquitin is expressed either as polyubiquitin or as a fusion with ribosomal proteins. Conjugation requires activating (E1) and conjugating (E2) enzymes that form thioesters (S) with the modifiers. Modification of cullins by RUB involves SCF(SKP1/cullin-1/F-box protein) /CBC(cullin-2/elongin B/elonginC) -like E3 enzymes that are also involved in ubiquitination. In contrast to ubiquitin, the UBLs do not seem to form multi-UBL chains. UCRP(IGS15) resembles two ubiquitin moieties linked head-to-tail. Whether HUB1 functions as a modifier is currently unclear. APG12 and URM1 are distinct from the other modifiers because they are unrelated in sequence to ubiquitin. Data contributed by S.Jentsch.

Western Blot

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.



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