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Product datasheet for R1193

ATG12 Rabbit Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	ELISA, WB
Recommended Dilution:	This purified polyclonal antibody reacts with yeast APG12 by Western blot and ELISA. This antibody using the specified conditions may recognize other prominent intrinsic bands (UBLs or conjugates). Other intrinsic bands are readily detectable at lower dilutions. A 22.1 kDa band corresponding to yeast APG12 is detected. Most yeast cell lysates can be used as a positive control without induction or stimulation. Recommended Dilutions: ELISA: 1/1,000-1/5,000. Western Blot: 1/200. Although not tested, this antibody is likely functional in Immunohistochemistry and Immunoprecipitation.
Reactivity:	Saccharomyces cerevisiae, Yeast
Host:	Rabbit
Clonality:	Polyclonal
Immunogen:	This purified antibody was prepared from rabbit serum after repeated immunizations with recombinant yeast APG12 protein.
Specificity:	Assay by immunoelectrophoresis resulted in a single precipitin arc against anti-Rabbit Serum.
Formulation:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2 with 0.01% (w/v) Sodium Azide as preservative State: Purified State: Lyophilized purified Ig fraction
Reconstitution Method:	Restore with 0.1 ml of deionized water or equivalent.
Concentration:	lot specific
Purification:	A multi-step process which includes Delipidation, Salt Fractionation and Ion exchange chromatography followed by extensive dialysis against the buffer stated below.
Conjugation:	Unconjugated

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Storage:	Store vial at 2-8°C prior to restoration. For extended storage add glycerol to 50% and then aliquot contents and freeze at -20°C or below. Centrifuge product if not completely clear after standing at room temperature. This antibody is stable for one month at 2-8°C as an undiluted liquid. Dilute only prior to immediate use. Avoid repeated freezing and thawing.
Stability:	Shelf life: One year from despatch.
Database Link:	P38316
Background:	Ubiquitin-like proteins 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. In yeast, autophagy, the delivery of cytoplasmic components to the lysosome/vacuole for degradation, requires a ubiquitin-like protein conjugation system, in which Apg12 is covalently bound to Apg12-Apg5 and Apg16.
Synonyms:	APG12L, Autophagy-related protein 12, APG12-like

Product images:

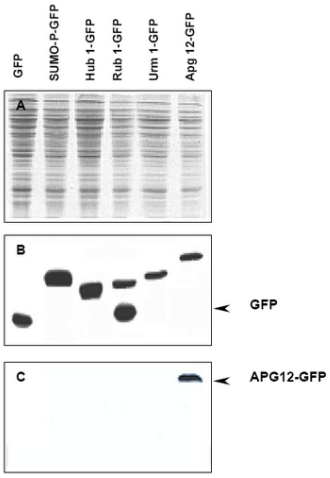

Figure 1. Immunoblot of APG12 fusion protein. Anti-APG12 antibody generated by immunization with recombinant yeast APG12 was tested by immunoblot against yeast lysates expressing the APG12-GFP fusion protein and other UBL fusion proteins. All UBLs possess limited homology to Ubiquitin and to each other, therefore it is important to know the degree of reactivity of each antibody against each UBL. Panel A shows total protein staining using ponceau. Panel B shows positions of free GFP or GFP containing recombinant proteins present in each lysate preparation after reaction with a 1:1,000 dilution of anti-GFP followed by reaction with a 1:15,000 dilution of HRP Donkey-a-Goat IgG MX. Panel C shows specific reaction with APG12 using a 1:2,000 dilution of IgG fraction of Rabbit-anti-APG12 (Yeast) followed by reaction with a 1:15,000 dilution of HRP Goat-a-Rabbit IgG MX. All primary antibodies were diluted in TTBS buffer supplemented with 5% non-fat milk and incubated with the membranes overnight at 4°C. Yeast lysate proteins were separated by SDS-PAGE using a 15% gel. This data indicates that anti-APG12 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, Lifesensors Inc., personal communication.

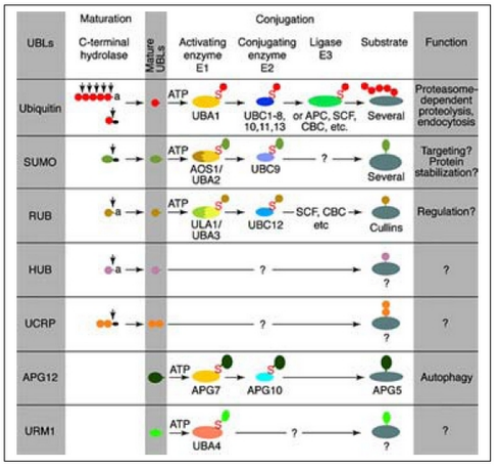

Figure 2. Conjugation pathways for ubiquitin and ubiquitin-like modifiers (UBLs). Most modifiers mature by proteolytic processing from inactive precursors (a; amino acid). Arrowheads point to the cleavage sites. Ubiquitin is expressed either as polyubiquitin or as a fusion with ribosomal proteins. Conjugation requires activating (E1) and conjugating (E2) enzymes that form thiolesters (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 (ISG15) 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, see reference 6.