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Product datasheet for R1194

HUB1 Rabbit Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	ELISA, WB
Recommended Dilution:	This purified polyclonal antibody reacts with yeast Hub1 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 9.7 kDa band corresponding to yeast Hub1 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: 1/500-1/2,000. Although not tested, this antibody is likely functional in Immunohistochemistry and Immunoprecipitation.
Reactivity:	Yeast
Host:	Rabbit
Clonality:	Polyclonal
Immunogen:	This purified antibody was prepared from rabbit serum after repeated immunizations with recombinant yeast Hub1 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:	Q6Q546
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. Hub1 ("Homologous to UBiquitin") may function as a modifier (see figure 2) but its role is unclear because it lacks the double glycine motif characteristic for ubiquitin and ubiquitin-like modifiers. Recently cell polarity factors Sph1 and Hbt1 have been identified as in vivo targets of Hub1 conjugation. HUB1 has close homologs in other species including humans. The human homologs is called Ubl5.
Synonyms:	YNR032C-A

Product images:

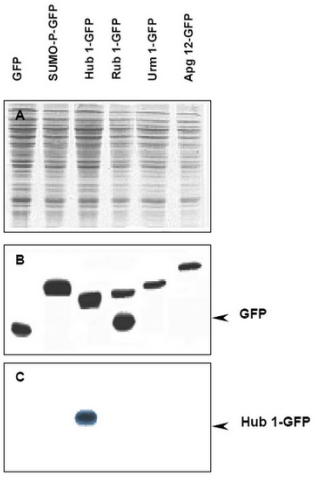

Figure 1. Immunoblot of Hub1 fusion protein. Anti-Hub1 antibody generated by immunization with recombinant yeast Hub1 was tested by immunoblot against yeast lysates expressing the Hub1-GFP fusion protein and other UBL fusion proteins. All UBLs possess limited homology to Ubiquitin and to each other, 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 Hub1 using a 1:500 dilution of IgG fraction of Rabbit-anti-Hub1 (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-Hub1 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.

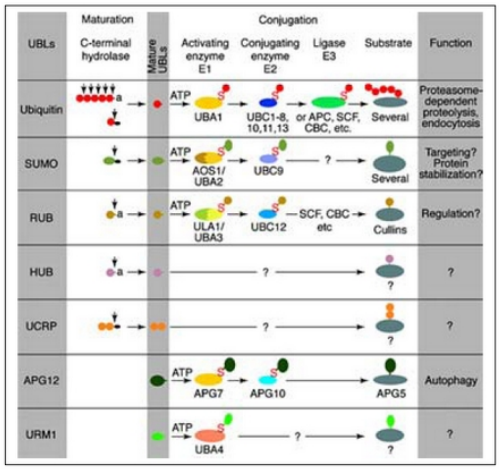

Figure 2. Conjugation pathways for ubiquitin and ubiquitin-like modifiers (UBLs). Most modifiers mature by proteolytic processing from inactive precursors (a; amino acid). Arrowheads point to the cleavage sites. Ubiquitin is expressed either as polyubiquitin or as a fusion with ribosomal proteins. Conjugation 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 4.