

Datasheet for 200-401-442**Hub1 Antibody****Overview**

Description:	Anti-Hub1 (RABBIT) Antibody - 200-401-442
Item No.:	200-401-442
Size:	500 µg
Applications:	WB
Reactivity:	Yeast
Host Species:	Rabbit

Product Details

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.
Synonyms:	rabbit anti-Hub1 Antibody, rabbit anti-Hub 1 Antibody, Homologous to Ubiquitin antibody, Hub1 protein antibody, HUB1 target protein 1 antibody, Hub1p antibody, Ubiquitin like protein modifier antibody
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	HUB1
Reactivity:	Yeast
Immunogen Type:	Recombinant Protein

Immunogen:	This purified antibody was prepared from rabbit serum after repeated immunizations with recombinant yeast Hub1 protein.
Purity/Specificity:	This product 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 - Q6Q546 • NCBI - 6324360 • GeneID - 855767

Application Details

Tested Applications:	WB
Application Note:	This purified polyclonal antibody reacts with yeast Hub1 by western blot and ELISA. Although not tested, this antibody is likely functional in immunohistochemistry and immunoprecipitation. This antibody using the specified conditions may recognize other prominent intrinsic bands (UBLs or conjugates). Other intrinsic bands are readily detectable at lower dilutions. For immunoblotting 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.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:1,000 - 1:5,000
WB:	1:500 - 1:2,000

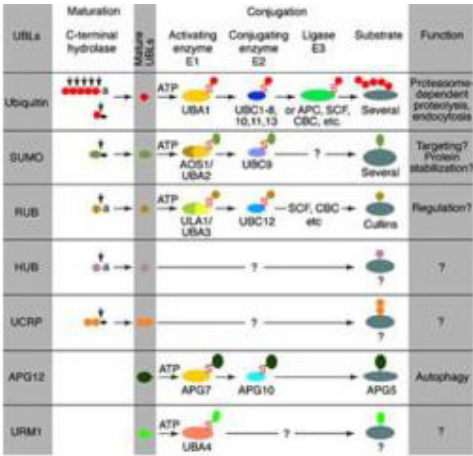
Formulation

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

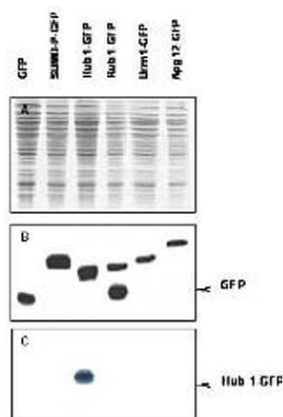
Shipping Condition:	Ambient
Storage Condition:	Store vial at 4° C prior to restoration. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for 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 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.



Western Blot

Western blot of Hub1 fusion protein. Anti-Hub1 antibody generated by immunization with recombinant yeast Hub1 was tested by western blot 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 Rockland's anti-GFP (code # 600-101-215) followed by reaction with a 1:15,000 dilution of HRP Donkey-a-Goat IgG MX (code # 605-703-125). Panel C shows specific reaction with Hub1 using a 1:500 dilution of Rockland's IgG fraction of Rabbit-anti-Hub1 (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. 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.

Disclaimer

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