

Certificate of Analysis

Product: IgG fraction of Anti-Rub1 (Yeast) (Rabbit)

Code: 200-401-427

Lot # 19054

Size: 500 µg

Physical State: Lyophilized

Antibody Concentration: 1.0 mg/ml (by UV absorbance at 280 nm)

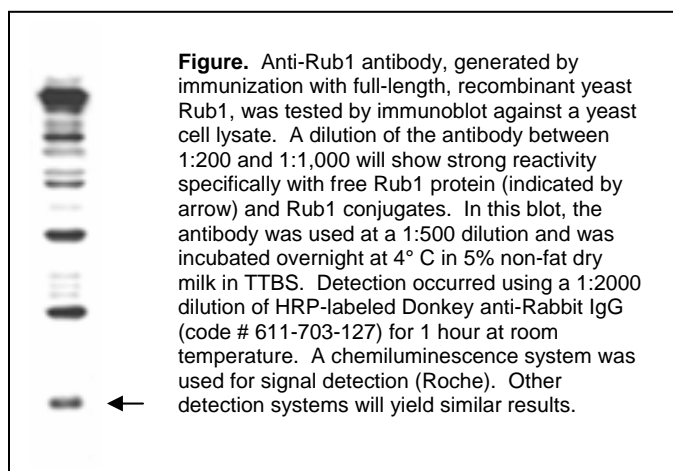
Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Stabilizer: None

Preservative: 0.01% (w/v) Sodium Azide

Storage Conditions: Store vial at 4° C prior to restoration. Restore with 0.5 ml of deionized water (or equivalent). 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 date is one (1) year from date of restoration.

Background Information: Ubiquitin-like proteins fall into two classes: the first class, ubiquitin-like modifiers (UBL's) function as modifiers in a manner analogous to that of ubiquitin. Examples of UBL's are SUMO, Rub1 (also called Nedd8), Apg12, and Hub1. Proteins of the second class include parkin, RAD23, and DSK2, and are designated ubiquitin-domain proteins (UDP's). These proteins contain domains that are related to ubiquitin but are otherwise unrelated to each other. In contrast to UBL's, UDP's are not proteolytically processed or conjugated to other proteins. Rub1, and the corresponding human homolog Nedd8, are activated by the E1 ubiquitin-activating enzyme UBA2, which forms isopeptide linkages between thioesters. Nedd8 shows 80% homology to ubiquitin. The best known targets of Rub1 modification are members of the cullin family. Cullins are subunits of an E3-ubiquitin ligase complex called the Skp1/Cul1/Cdc53-F-box (SCF). The SCF promotes transfer of ubiquitin from a ubiquitin conjugating enzyme (E2) to the target protein. Rub1 modification may regulate SCF function or localization.



Application Note(s): This purified polyclonal antibody reacts with yeast Rub1 by western blot and ELISA. Although not tested, this antibody is likely functional in immunohistochemistry and immunoprecipitation.

Recommended Dilution(s): For immunoblotting, a 1:500 dilution is recommended. A 6 kDa band corresponding to yeast Rub1 is detected. Most yeast cell lysates can be used as a positive control without induction or stimulation. For ELISA, a 1:1,000 to 1:5,000 dilution is recommended. Researchers should determine optimal titers for other applications.

Purity and 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.

Immunogen: This purified antibody was prepared from rabbit serum after repeated immunizations with full-length recombinant yeast Rub1 protein.

Reference(s):

del Pozo JC, Dharmasiri S, Hellmann H, Walker L, Gray WM, Estelle M (2002) AXR1-ECR1-dependent conjugation of RUB1 to the Arabidopsis Cullin AtCUL1 is required for auxin response. *Plant Cell*, **14**(2): 421-33.

Wang X, Kang D, Feng S, Serino G, Schwechheimer C, Wei N (2002) CSN1 N-terminal-dependent activity is required for Arabidopsis development but not for Rub1/Nedd8 deconjugation of cullins: a structure-function study of CSN1 subunit of COP9 signalosome. *Mol. Biol. Cell*, **13**(2):646-55.

del Pozo JC, Estelle M (2000) The Arabidopsis cullin AtCUL1 is modified by the ubiquitin-related protein RUB1. *Proc. Natl. Acad. Sci. USA*, **96**(26):15342-7.

Kamura T, Conrad MN, Yan Q, Conaway RC, Conaway JW (2000) The Rbx1 subunit of SCF and VHL E3 ubiquitin ligase activates Rub1 modification of cullins Cdc53 and Cul2. *Genes Dev.*, **13**(22):2928-33.

del Pozo JC, Timpte C, Tan S, Callis J, Estelle M (1998) The ubiquitin-related protein RUB1 and auxin response in Arabidopsis. *Science*, **280**(5370):1760-3.

Liakopoulos D, Busgen T, Brychzy A, Jentsch S, Pause A (1999) Conjugation of the ubiquitin-like protein NEDD8 to cullin-2 is linked to von Hippel-Lindau tumor suppressor function. *Proc. Natl. Acad. Sci. USA*, **96**(10):5510-5.

USDA Certification: All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation.

Note: This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information.