

Rabbit (polyclonal) Anti-ROC1 Unconjugated

PRODUCT ANALYSIS SHEET

Catalog Number: AHO0402

Lot Number: See product label

Quantity/Volume: 0.5 mg/0.5 mL

Form of Antibody: Purified immunoglobulins in phosphate buffered saline, pH 7.4, with 1.0% bovine serum

albumin.

Preservation: 0.1% sodium azide (Caution: sodium azide is a poisonous and hazardous substance.

Handle with care and dispose of properly.)

Purification: Purified from rabbit serum by Protein G chromatography.

Immunogen: A synthetic peptide corresponding to amino acid residues 97-108 (Cys-

PLDNREWEFQKYGH) at the N-terminus of human ROC1 coupled to KLH.

Specificity: This antibody recognizes a protein of 15 kDa, identified as ROC1. ROC1, also called

Rbx1 or Hrt1, is a RING finger protein that is homologous to APC11, a subunit of the anaphase-promoting complex. ROC1 commonly interacts with all cullins and is one of the subunits of the SCF ubiquitin protein ligases. In yeast, ROC1 encodes an essential gene whose expression results in multiple, elongated buds and accumulation of Sic1p and Cln2p. ROC1 immunocomplexes can catalyze isopeptide ligations to form polyubiquitination chains in an E1- and E2-dependent manner. For example, phosphorylated IκBα ubiquitination can be catalyzed by the ROC1 immunocomplex *in vitro*. ROC1 mutations completely abolish their ligase activity without noticeable

changes in associated proteins.

Species Reactivity: Human and mouse ROC1 protein. Other species were not tested.

Applications: This antibody is suitable for use in immunoprecipitation and Western blotting. This

antibody has been reported to co-immunoprecipitate associated cullin and ubiquitin

ligase activity.

Suggested Working

Dilutions:

For immunoprecipitation, use $1-2~\mu g$ of antibody per $200-500~\mu g$ of protein lysate;

and for Western blotting, a 1:1000-1:1500 dilution is recommended.

Recommended Positive

Control:

PI AHO0402

HeLa, CTLL and NIH-3T3 cells.

Storage: Store at 2-8°C. For long term storage, aliquot into small volumes and store at -20°C.

Avoid repeated freeze-thaw cycles to prevent denaturing the antibody.

This product is for research use only. Not for use in diagnostic procedures.

www.invitrogen.com

Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288 • E-mail: techsupport@invitrogen.com

References:

Seol, H. -J., et al. (1999) Cdc53/cullin and the essential Hrt1 RING-H2 subunit of SCF define a ubiquitin ligase module that activates the E2 enzyme Cdc34. Genes Dev. 13:1614-1626.

Ohta, T., et al. (1999) ROC1, a homolog of APC11, represents a family of cullin partners with an associated ubiquitin ligase activity. Mol. Cell 3(4):535-41.

Tan, P., et al. (1999) Recruitment of a ROC1-CUL1 ubiquitin ligase by Skp1 and HOS to catalyze the ubiquitination of I kappa B alpha. Mol. Cell 3(4):527-33.

Iwai, K., et al. (1999) Identification of the von Hippel-Lindau tumor-suppressor protein as part of an active E3 ubiquitin ligase complex. Proc. Nat'l. Acad. Sci. USA. 96(22):12436-41.

Winston, J.T., et al. (1999) A family of mammalian F-box proteins. Curr. Biol. 9(20):1180-1182.



Figure: Proteins from CTLL cell extracts were reduced and resolved by SDS PAGE on a 4-20% Tris-glycine gel. The proteins were then transferred to PVDF. Membranes were incubated with a 1:1000 dilution of the anti-ROC1 antibody. After washing, membranes were incubated with goat F(ab')₂ anti-rabbit IgG alkaline phosphatase (ALI4405) and bands were detected using the Tropix WesternStarTM detection method.