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ProductInformation

Anti-ROC1
Developed in Rabbit, IgG Fraction of Antiserum

Product Number **R 4402**

Product Description

Anti-ROC1 (Regulator of Cullins) is developed in rabbit using a synthetic peptide corresponding to amino acid 97-108 in the N-terminal region of human ROC1 as the immunogen. The antiserum is purified using Protein G chromatography. Anti-ROC1 specifically recognizes a 15 kDa protein, identified as ROC1. The antibody detects human and mouse ROC1. It has been used in immunoblotting and immunoprecipitation applications.

ROC1, also known as RING-BOX 1 (Rbx1) or HRT1, is a highly conserved RING finger protein that is homologous to APC11, a subunit of the anaphase-promoting complex (APC). The protein is designated RING-BOX 1 because it contains a RING-H2 finger-like motif. The mouse and human Rbx1 proteins are identical, and there are homologs in *Drosophila*, *C. elegans* and *C. cervisiae*.[1] ROC1 commonly interacts with all cullins, which are proteins associated with transcriptional elongation and ubiquitination. ROC1 is a subunit and a potent activator of the SCF (Skp1-Cullin (Cdc53)-F-box protein) -cdc4 complex that is required for ubiquitination of the cyclin-dependent kinase inhibitor Sic1 and for G1 to S cell-cycle transition.[2] In yeast, ROC1 is encoded by an essential gene whose expression results in multiple, elongated buds and accumulation of Sic1p and Cln2p. ROC1 immunocomplexes can catalyze isopeptide ligations to form polyubiquitin chains. For example, phosphorylated IκBα ubiquitination can be catalyzed by the ROC1 immunocomplex *in vitro*.[3]

The crystal structure of the Cul1-Rbx1-Skp1-F box-Skp2 SCF ligase complex shows that the globular domain of Cul1 binds the RING finger protein Rbx1 through an intermolecular β-sheet. This forms a two-subunit catalytic core that recruits the ubiquitin-conjugating enzyme.[4] All cullins, through their binding with ROC proteins, constitute active ubiquitin ligases, suggesting the existence *in vivo* of a large number of cullin-RING ubiquitin ligases.[5]

Reagent

Anti-ROC1, at approximately 1 mg/ml, is supplied as a solution in phosphate buffered saline, pH 7.4, containing 1.0% BSA and 0.1% sodium azide. The amount of the reagent is sufficient for 10 blots.

Precautions and Disclaimer

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Storage/Stability

Store at –20 °C. For extended storage, upon initial thawing, freeze in working aliquots. Do not store in frost-free freezers. Avoid repeated freezing and thawing to prevent denaturing the antibody. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 6 months when stored appropriately.

Product Profile

A recommended working dilution of 1:1000 to 1:1500 is determined by immunoblotting using proteins from cell extracts of HeLa, CTLL or NIH-3T3 cells. For immunoprecipitation, the recommended working concentration is 1 to 2 µg of antibody per 200-500 µg of protein lysate. The antibody may co-immunoprecipitate associated cullin and ubiquitin ligase activity.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

References

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2. Ohta, T., et al., ROC1, a homolog of APC11, represents a family of cullin partners with an associated ubiquitin ligase activity. Mol. Cell, **3**, 535-541 (1999).

3. Tan, P., et al., Recruitment of a ROC1-CUL1 ubiquitin ligase by Skp1 and HOS to catalyze the ubiquitination of IκBα. Mol. Cell, **3**, 527-533 (1999).
4. Zheng, N., et al., Structure of the Cul1-Rbx1-Skp1-F box-Skp2 SCF ubiquitin ligase complex. Nature, **416**, 703-709, (2002).
5. Furukawa, M., et al., Activation of UBC5 ubiquitin-conjugating enzyme by the RING finger of ROC1 and assembly of active ubiquitin ligases by all cullins. J. Biol. Chem., **277**, 15758-15765 (2002).

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