

Certificate of Analysis

Product: Anti-Human Cul1 (C-terminal specific) [RABBIT]

Code: 100-401-A01

Lot #: 15443

Size: 100 µL

Physical State: Liquid (sterile filtered)

Protein Concentration: 85 mg/ml (by Refractometry)

Buffer: None

Stabilizer: None

Preservative: 0.01% (w/v) Sodium Azide

Storage Conditions: Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. 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 opening.

Background Information: Cullins assemble a potentially large number of ubiquitin ligases by binding to the RING protein ROC1 to catalyse polyubiquitination, as well as binding to various specificity factors to recruit substrates. Cullin 1 is an essential component of the SCF (SKP1-CUL1-F-box protein) E3 ubiquitin ligase complex, which mediates the ubiquitination of proteins involved in cell cycle progression, signal transduction and transcription. In the SCF complex, cul1 serves as a rigid scaffold that organizes the SKP1-F-box protein and RBX1 subunits. Cul1 may also contribute to catalysis through positioning of the substrate and the ubiquitin-conjugating enzyme. Cul1 is part of the SCF complex consisting of CUL1, RBX1, SKP1 and SKP2, where it interacts directly with SKP1, SKP2 and RBX1. Cul1 also interacts with RNF7 and is part of a complex with TIP120A/CAND1 and RBX1. The unneddylated form interacts with TIP120A/CAND1 and the interaction negatively regulates the association with SKP1 in the SCF complex.

Application Note(s): This antibody reacts with human Cul1 by immunohistochemistry, western blot and immunoprecipitation. The antibody immunoprecipitates *in vitro* translated product and protein from cell lysates (using HeLa or NIH-3T3). Do not IP in the presence of NP-40, but rather use 0.1% SDS. An 89.6 kDa band corresponding to human Cul1 is detected. Most cell lines expressing Cul1 can be used as a positive control. Researchers should determine optimal titers for other applications.

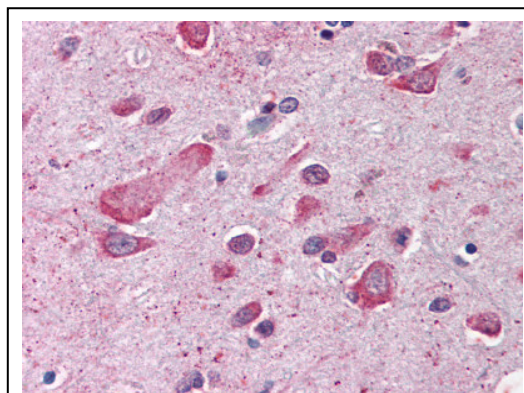


Figure 1. Immunohistochemistry. Rockland Immunochemicals' Anti-CUL1 antibody was diluted 1:500 to detect CUL1 in human brain cortex tissue. Tissue was formalin fixed and paraffin embedded. No pre-treatment of sample was required. The image shows the localization of antibody as the precipitated red signal, with a hematoxylin purple nuclear counter stain.

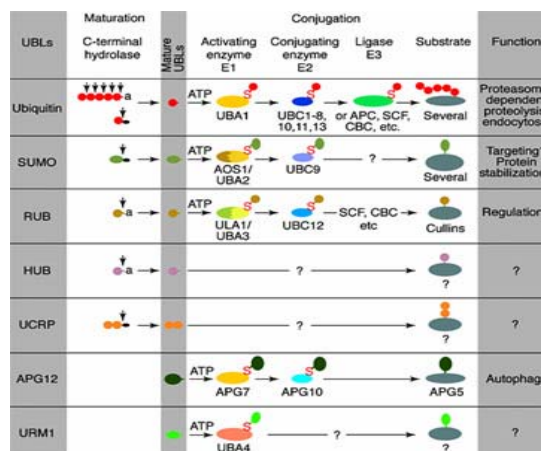


Figure 2. Conjugation pathways for ubiquitin and ubiquitin-like modifiers (UBLs). Most modifiers mature by proteolytic processing from inactive precursors (a; amino acid). Arrowheads point to the cleavage sites. Ubiquitin is expressed either as polyubiquitin or as a fusion with ribosomal proteins. Conjugation requires activating (E1) and conjugating (E2) enzymes that form thioesters (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(IG15) 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 references below.

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Recommended Dilution(s):	ELISA	1:2,000 - 1:10,000
	WESTERN BLOT	1:500 - 1:1,000
	IMMUNOHISTOCHEMISTRY	1:500
	OTHER APPLICATIONS	User Optimized

Purity and Specificity: This product is monospecific antiserum processed by delipidation and defibrination followed by sterile filtration. This product reacts with human Cullin 1. Cross reactivity is expected against mouse Cul1 based on sequence homology.

Immunogen: This antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to amino acids 742-752 of Human Cul1 (C-terminus) coupled to KLH. 766-776

Related Link(s): NCBI [NP_003583](#) Swis-Prot [Q13616](#)

Specific Reference(s):

Michel, J.J. and Xiong, Y. (1998) Human CUL-1, but not other cullin family members, selectively interacts with SKP1 to form a complex with SKP2 and cyclin A. *Cell Growth Differ.* **9** (6), 435-449.

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Ohta, T., Michel, J.J., Schottelius, A.J. and Xiong, Y. (1999) ROC1, a homolog of APC11, represents a family of cullin partners with an associated ubiquitin ligase activity. *Mol. Cell* **3** (4), 535-541.

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General Reference(s):

Hori, T., Osaka, F., Chiba, T., Miyamoto, C., Okabayashi, K., Shimbara, N., Kato, S. and Tanaka, K. (1999) Covalent modification of all members of human cullin family proteins by NEDD8. *Oncogene* **18** (48), 6829-6834.

Read, M.A., Brownell, J.E., Gladysheva, T.B., Hottelet, M., Parent, L.A., Coggins, M.B., Pierce, J.W., Podust, V.N., Luo, R.-S., Chau, V. and Palombella, V.J. (2000) Nedd8 modification of cul-1 activates SCF(beta-TrCP)-dependent ubiquitination of I kappa B alpha. *Mol. Cell Biol.* **20** (7), 2326-2333.

Tan, P., Fuchs, S.Y., Chen, A., Wu, K., Gomez, C., Ronai, Z. and Pan, Z.Q. (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), 527-533 (1999).

Jentsch S, Pyrowolakis G. (2000) Ubiquitin and its kin: how close are the family ties? *Trends Cell Biol.* **10**(8):335-42.

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#611-145-122	DyLight™ 680 Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (GOAT) MX10
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