ROCKLAND
Immunochemicals for Research
800-656-7625
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Certificate of Analysis

Product: Anti-Human Cul2 (C-terminal specific) [RABBIT]

Code: 100-401-A02

Lot #: 15410

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 2 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, cul2 serves as a rigid scaffold that organizes the SKP1-F-box protein and RBX1 subunits. Cul2 may also contribute to catalysis through positioning of the substrate and the ubiquitin-conjugating enzyme. Cul2 is part of the SCF complex consisting of CUL1, RBX1, SKP1 and SKP2, where it interacts directly with SKP1, SKP2 and RBX1. Cul2 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 Cul2 by immunohistochemistry, western blot and immunoprecipitation. The antibody immunoprecipitates *in vitro* translated product and protein from cell lysates (using HeLa or NIH-3T3). An 86.9 kDa band corresponding to human Cul2 is detected. Most cell lines expressing Cul2 can be used as a positive control. Researchers should determine optimal titers for other applications.

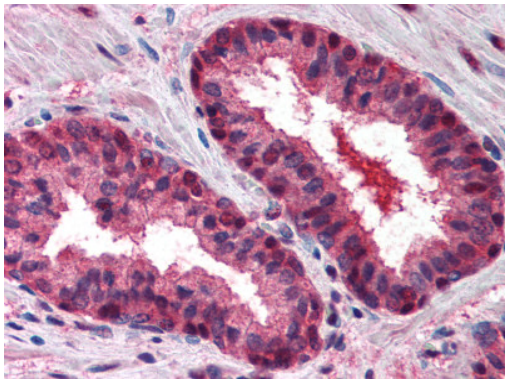

Figure 1. Immunohistochemistry. Rockland Immunochemicals' Anti-CUL2 antibody was diluted 1:500 to detect CUL2 in human prostate tissue. Tissue was formalin fixed and paraffin embedded. No pre-treatment of sample was required. The image shows the localization of antibody as the precipitated red signal, with a hematoxylin purple nuclear counter stain.

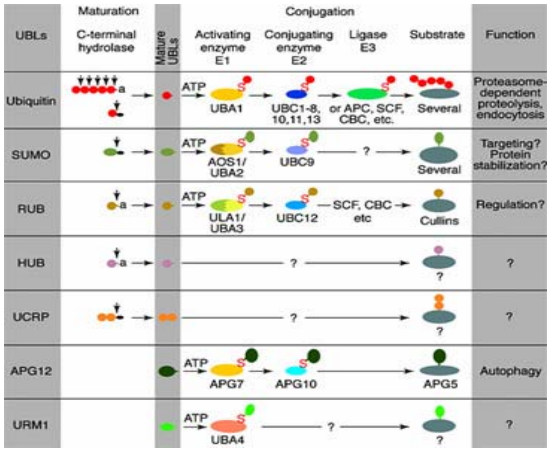

Figure 2. Conjugation pathways for ubiquitin and ubiquitin-like modifiers (UBLs). Most modifiers mature by proteolytic processing from inactive precursors (a; amino acid). Arrowheads point to the cleavage sites. Ubiquitin is expressed either as polyubiquitin or as a fusion with ribosomal proteins. Conjugation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

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 2. Cross reactivity with Cul2 from other sources has not been determined.

Immunogen: This antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to amino acids 733-745 of Human Cul2 (C-terminus) coupled to KLH.

Related Link(s): Accession # NP 003582

Specific Reference(s):

Feng, H., Zhong, W., Punkosdy, G., Gu, S., Zhou, L., Seabolt,. E.K. and Kipreos, E.T. (1999) CUL-2 is required for the G_1-to S-phase transition and mitotic chromosome condensation in Caenorhabditis elegans. *Nat Cell Bio* **1**, 486-492.

Kipreos,E.T., Lander,L.E., Wing,J.P., He,W.W. and Hedgecock,E.M. (1996) cul-1 is required for cell cycle exit in C. elegans and identifies a novel gene family. *Cell* 85 (**6**), 829-839.

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.

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.

Mathias, N., Johnson, S.L., Winey, M., Adams, A.E., Goetsch, L., Pringle, J.R., Byers, B., Goebl, M.G. (1996) Cdc53p acts in concert with Cdc4p and Cdc34p to control the G1-to-S-phase transition and identifies a conserved family of proteins. *Mol Cell Biol* **16**:12, 6634-6643.

General Reference(s):

Kamura,T., Sato,S., Iwai,K., Czyzyk-Krzeska,M., Conaway,R.C. and Conaway,J.W. (2000) Activation of HIF1alpha ubiquitination by a reconstituted von Hippel-Lindau (VHL) tumor suppressor complex. *Proc. Natl. Acad. Sci. U.S.A*. **97** (19), 10430-10435.

Wada,H., Yeh,E.T. and Kamitani,T. (1999) Identification of NEDD8-conjugation site in human cullin-2. *Biochem. Biophys. Res. Commun*. **257** (1), 100-105.

Pause,A., Lee,S., Worrell,R.A., Chen,D.Y., Burgess,W.H., Linehan,W.M. and Klausner,R.D. (1997) The von Hippel-Lindau tumor-suppressor gene product forms a stable complex with human CUL-2, a member of the Cdc53 family of proteins. *Proc. Natl. Acad. Sci. U.S.A*. **94** (6), 2156-2161.

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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#BSA-30	30% BOVINE SERUM ALBUMIN SOLUTION in 0.85% sodium chloride (no preservative or stabilizer) (500 ml)
#B304	NORMAL GOAT SERUM (NGS) (10 ml)
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#MB-070	Blocking Buffer for Fluorescent Western Blotting