

# Cul2/Rbx1 [untagged]

## E3 Ligase

Alternate Names: Cul2=Cullin2  
Rbx1 = HRT1, Regulator of cullins 1, Ring finger protein 75, RNF75, ROC1, ZYP protein

Cat. No. 63-1004-025  
Lot. No. 30215

Quantity: 25 µg  
Storage: -70°C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS



CERTIFICATE OF ANALYSIS Page 1 of 2

## Background

The enzymes of the ubiquitylation pathway play a pivotal role in a number of cellular processes including the regulated and targeted proteasome-dependent degradation of substrate proteins. Three classes of enzymes are involved in the process of ubiquitylation; activating enzymes (E1s), conjugating enzymes (E2s) and protein ligases (E3s). Cullin-RING-Ligases (CRLs) are one of the largest classes of ubiquitin E3 ligases and the enzymes of the NEDDylation pathway play a pivotal role in the activation of these CRLs. Akin to ubiquitylation, the E1 activating enzyme (APP-BP1/UBA3 heterodimer) and the E2 conjugating enzymes (UBE2M or UBE2F) are involved in mammalian NEDDylation of the Cullin Ring Ligases (CRLs) (Meyer-Schaller *et al.*, 2009; Huang *et al.*, 2011; Morimoto *et al.*, 2003). The human Cullin1-5 genes were first described by Kipreos *et al.* (1996). Cullin RING ligases (CRL) comprise the largest subfamily of ubiquitin ligases which are activated by Neddyl-ation. CRLs are involved in cell cycle regulation, DNA replication and the DNA damage response (DDR). CRLs consist of several subunits including, a scaffold protein (cullin) and a Ring finger protein; either Rbx1 (Cul1-4) or Rbx2 (Cul5) that binds a ubiquitin E2 Ube2M or Ube2F respectively (Sarikas *et al.*, 2011; Skowrya *et al.*, 1997). The first CRL to be identified was named Skp1/Cullin or Cdc53/F-box (SCF) from *Saccharomyces cerevisiae*. Many CRL E3 ligases have additional linker proteins such as Elongin B/C associated with Cul2 and DDB1 associated with Cul4. The Elongin B/C-Cul2 or Cul5-SOCS box (ECS) family also be-

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## Physical Characteristics

**Species:** human

**Source:** Insect (Sf21)

**Quantity:** 25 µg

**Concentration:** 0.5 mg/ml

**Formulation:** 50 mM HEPES pH 7.5,  
150 mM sodium chloride,  
2 mM dithiothreitol, 10% glycerol

**Molecular Weight:**  
Cul2: ~87.1 kDa; Rbx1: ~12.3 kDa

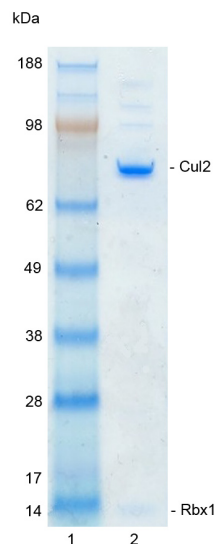
**Purity:** >75% by InstantBlue™ SDS-PAGE

**Stability/Storage:** 12 months at -70°C;  
aliquot as required

**Protein Sequences:** Please see page 2

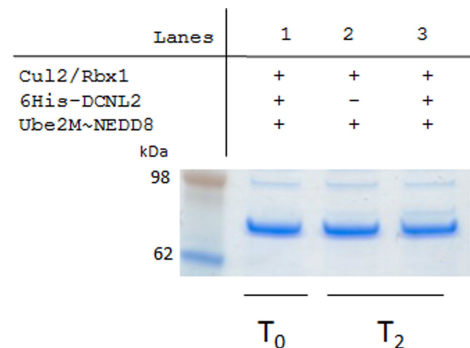
## Quality Assurance

**Purity:**  
4-12% gradient SDS-PAGE  
InstantBlue™ staining  
Lane 1: MW markers  
Lane 2: 1 µg Cul2/Rbx1



**Protein Identification:**  
Confirmed by mass spectrometry.

**E3 Ligase Assay:** The activity of Cul2/Rbx1 was validated indirectly through its ability to act as a substrate for Neddyl-ation in the presence of the NEDD8 E3 ligase His-DCNL2 and thioester-loaded His-UBE2M-NEDD8. Incubation of Cul2/Rbx1 and thioester loaded His-UBE2M-NEDD8 in the presence or absence of His-DCNL2 at 4°C was compared at two time points T<sub>0</sub> and T<sub>2</sub> minutes. Neddyl-ation of the Cul2 subunit in the presence of His-DCNL2 was demonstrated.



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CERTIFICATE OF ANALYSIS Page 2 of 2

## Background

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longs to the CRL superfamily (Kile *et al.*, 2002). SCF and ECS ubiquitin ligases have structural similarities in that both contain Rbx1 or Rbx2 as a RING finger protein and Cul1, Cul2 or Cul5 as a scaffold protein (Kile *et al.*, 2002; Kamura *et al.*, 2004). The von Hippel-Lindau (VHL) complex is a ubiquitin ligase which targets the Hypoxia Inducible Factor alpha (HIFα) family of transcription factors for proteasomal degradation. The complex comprises pVHL, the Cul2/Rbx1 subunit and the BC box protein Elongin B/C. Loss of functional pVHL protein prevents the oxygen dependent degradation of HIF1α resulting in constitutive expression of HIF dependent genes and consequently VHL disease (Okumura *et al.*, 2012).

### References:

Huang G, Kaufman A J, Ramanathan Y, Singh B (2011) SCCRO (DCUN1D1) promotes nuclear translocation and assembly of the neddylation E3 complex. *J Biol Chem* **286**, 10297-10304.

Kamura T, Maenaka K, Kotoshiba S, Matsumoto M, Kohda D, Conaway RC, Conaway JW, Nakayama KI (2004) VHL-box and SOCS-box domains determine binding specificity for Cul2-Rbx1 and Cul5-Rbx2 modules of ubiquitin ligases. *Genes Dev* **18**, 3055-65.

Kile BT, Schulman BA, Alexander WS, Nicola NA, Martin HM, Hilton DJ (2002) The SOCS box: a tale of destruction and degradation. *Trends Biochem Sci* **27**, 235-41.

Kipreos ET, Lander LE, Wing JP, He WW, Hedgecock EM (1996) cul-1 is required for cell cycle exit in *C. elegans* and identifies a novel gene family. *Cell* **85**, 829-839.

Meyer-Schaller N, Chou YC, Sumara I, Martin DD, Kurz T, Katheder N, Hofmann K, Berthiaume LG, Sicheri F, Peter M. (2009) The human Dcn1-like protein DCNL3 promotes Cul3 neddylation at membranes. *Proc Natl Acad Sci U S A* **106**, 12365-12370.

Morimoto M, Nishida T, Nagayama Y, Yasuda H (2003) Neddylation of Cul1 is promoted by Roc1 as a Neddylation E3 ligase and regulates its stability. *Biochem Biophys Res Commun* **301**, 392-398.

Okumura F, Matsuzaki M, Nakatsukasa K, Kamura T (2012) The Role of Elongin BC-Containing Ubiquitin Ligases. *Front Oncol* **2**, 1-13.

Skowrya D, Craig KL, Tyers M, Elledge SJ, Harper JW (1997) F-box proteins are receptors that recruit phosphorylated substrates to the SCF ubiquitin-ligase complex. *Cell* **91**, 209-219.

## Physical Characteristics

Continued from page 1

### Protein Sequence: Cullin 2

GSMSLKPRVDFDETWNKLLTTIKAVVM  
LEYVERATWNDRFSDIALCVAPEPLGER  
LYTETKIFLENHVRHLHKRVLESEEQV  
LVMHRYWEEYSKGADMDCLYRLNTQFIK  
KNKLTEADLQYGGVDMNEPLMEIGELALD  
MWRKLMVEPLQAILRMLLREIKNDRGGEDP  
NQKVIHGVINSFVHVEQYKKKFPLKFYQEIF  
ESPFLTETGEYKQEASNLLQESNCSQYMEKV  
LGRLKDEEIRCRKYLHPSSYTKVIHECQQRM  
VADHLQFLHAECHNIIRQEKKNDMANMYVLL  
RAVSTGLPHMIQELQNHIHDEGLRATSNLTQEN  
MPTLFVESVLEVHGKFVQLINTVLNGDQHF  
MSALDKALTSVVNYREPKSVCKAPELLAKY  
CDNLLKKSAKGMTENEVEDRLTSFITVFKYID  
DKDVFQKFYARMLAKRLIHGLSMSMDSEEAM  
INKLKQACGYEFTSKLHRMYTDMSVSADLNN  
KFNNFIKNQDTVIDLGISFQIYVLQAGAW  
PLTQAPSSTFAIPQELEKSVQMFELFYSQHFS  
GRKLTWLHYLCTGEVKMNYLGKPYVAMVTTYQ  
MAVLLAFNSETVSYKELQDSTQMNEKELTK  
TIKSLLDVKMINHDSEKEDIDAESSFSLNMNF  
SSKRTKFKITTSMQKDTPQEMEQTRSAVDEDRK  
MYLQAAIVRIMKARKVLRHNALIQEVISQS  
RARFNPSISMIKKCIEVLIDKQYIERSQASA  
DEYSYVA

The residues underlined remain after cleavage and removal of the purification tag.

Cullin2 (regular text): Start ***bold italics*** (amino acid residues 1-745)

Accession number: AAH09591.1

Cullin2 [Dac tagged] / Rbx1 was cleaved with TEV protease [6His tagged]. The Dac tag and TEV protease [6His-Tagged] were removed by capturing on amp sepharose and nickel resin respectively.

### Protein Sequence: Rbx1

MAAAMDVDTPSGTNSGAGKKRFEVKKW  
NAVALWADIVVDNCAICRNHIMDLCIEC  
QANQASATSEECTVAWGVCNHAFHFHCISR  
WLKTRQVCPLDNREWEFQKYGH

Rbx1 (regular text): Start ***bold italics*** (amino acid residues 1-108)

Accession number: NP\_055063.1



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