Cul3/Rbx1 [untagged]

E3 Ligase

Alternate Names: Cul3 = KIAA0617
Rbx1=HRT1, Regulator of cullins 1, Ring finger protein 75, RNF75, ROC1, ZYP protein

Cat. No. **63-1003-025** Quantity: 25 µg
Lot. No. **30206** Storage: -70˚C

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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 largest class of ubiquitin E3 ligases and the enzymes of the NEDDylation pathway play a pivotal role in the activation of these, 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 Neddylation. CRLs are involved in cell cycle regulation, DNA replication, DNA damage response (DDR). CRLs contain subunits including, a scaffold protein (cullin family protein), a Ring finger protein either Rbx1 (Cul1-4) or Rbx2 (Cul5) that binds a ubiquitin E2 Ube2M or Ube2F respectively (Sarikas *et al.*, 2011; Skowyra *et al.*, 1997). Cul3 expression in human fibroblasts is induced by phorbol 12-myristate 13-acetate (PMA) and

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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:
Cul3: ~89.0 kDa; Rbx1: ~12.3 kDa

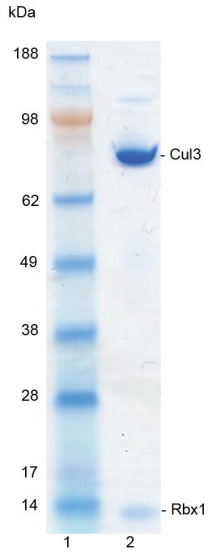
Purity: >95% by InstantBlue™ SDS-PAGE

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

Protein Sequences: Please see page 2

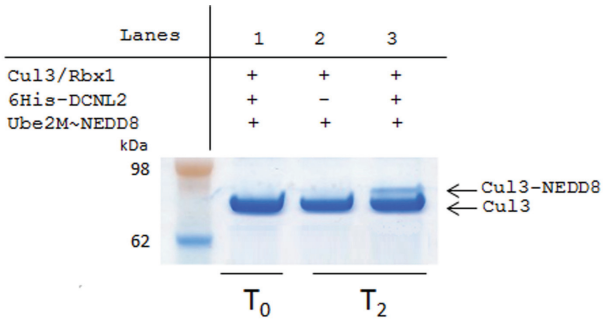
Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg Cul3/Rbx1



Protein Identification:
Confirmed by mass spectrometry.

E3 Ligase Assay: The activity of Cul3/Rbx1 was validated indirectly through its ability to act as a substrate for neddylation in the presence of the NEDD8 E3 ligase His-DCNL2 and thioester-loaded His-Ube2M~NEDD8. Incubation of Cul3/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_0 and T_2 minutes. Neddylation of the Cul3 subunit in the presence of His-DCNL2 was demonstrated.



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Lot-specific COA version tracker: v1.0.1

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Background

Continued from page 1

suppressed by salicylate (Du *et al.*, 1998). The Cul3/Kelch like 9 (KLHL9) /Kelch like 13 (KLHL13) complex is an E3 ligase that controls the behavior of Aurora B on mitotic chromosomes and thereby coordinating mitotic progression and completion of cytokinesis (Sumara *et al.*, 2007). Interaction of Cul3 with Kelch like 7 (KLHL7) leads to the ubiquitylation of the dopamine receptor D4 (DRD4) (Rondou *et al.*, 2008).

References:

Du M, Sansores-Garcia L, Zu Z, Wu KK, (1998) Cloning and expression analysis of a novel salicylate suppressible gene, Hs-CUL-3, a member of cullin/Cdc53 family. *J Biol Chem* **273**, 24289-24292.

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

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) Nedd8-modification of Cul1 is promoted by Roc1 as a Nedd8-E3 ligase and regulates its stability, *Biochem Biophys Res Commun* **301**, 392-398.

Rondou P, Haegeman G, Vanhoenacker P, Van Craenenbroeck K, (2008) BTB protein KLHL12 targets the dopamine D4 receptor for ubiquitination by a Cul3-based E3 ligase. *J Biol Chem* **283** 11083-11096.

Sarikas, A, Hartmann, T and Pan, ZQ (2011) The cullin protein family, *Genome Biology* 12, 220.

Skowyra 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.

Sumara I, Quadroni M, Frei C, Olma MH, Sumara G, Ricci R, Peter MA (2007) Cul3-based E3 ligase removes Aurora B from mitotic chromosomes, regulating mitotic progression and completion of cytokinesis in human cells. *Dev Cell* **12**, 887-900.

Physical Characteristics

Continued from page 1

Protein Sequence: Cullin 3

GGS***M***SNLSKGTGSRKDTKMRIRAFPMTM
DEKYVNSIWDLLKNAIQEIQRKNNSGLS
FEELYRNAYTMVLHKHGEKLYTGLREVVTE
HLINKVREDVLNSLNNNFLQTLNQAWND
HQTAMVMIRDILMYMDRVYVQQNNVENVYNL
GLIIFRDQVVRYGCIRDHLRQTLLDMI
ARERKGEVVDRGAIRNACQMLMILGLEGRS
VYEEDFEAPFLEMSAEFFQMESQKFLAEN
SASVYIKKVEARINEEIERVMHCLDKSTEEP
IVKVVERELISKHMKTIVEMENSGLVHM
LKNGKTEDLGCMYKLFSRVPNGLKTMCECMS
SYLREQGKALVSEEGEGKNPVDYIQGLLDLK
SRFDRFLLESFNNDRLFKQTIAGDFEYFLN
LNSRSPEYLSLFIDDKLKKGVKGLTEQE
VETILDKAMVLFRFMQEKDVFERYYKQHLAR
RLLTNKSVSDDSEKNMISKLKTECGC
QFTSKLEGMFRDMSISNTTMDEFRQHLQAT
GVSLGGVDLTVRVLTTGYWPTQSATPKC
NIPPAPRHAFEIFRRFYLAKHSGRQLTLQH
HMGSADLNATFYGPVKKEDGSEVGVG
GAQVTGSNTRKHILQVSTFQMTILMLFN
NREKYTFEEIQQETDIPERELVRALQS
LACGKPTQRVLTKEPKSKEIENGHIFT
VNDQFTSKLHRVKIQTVAAKQGESDPERKET
RQKVDDDRKHEIEAAIVRIMKSRKKMQHNVL
VAEVTQQLKARFLPSPVVIKKRIEGLIEREY
LARTPEDRKVYTYVA

The residues underlined remain after cleavage and removal of the purification tag.
Cullin3 (regular text): Start ***bold italics*** (amino acid residues 1-768)
Accession number: NP_003581.1

Cullin3 [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
NAVALWAWDIVVDNCAICRNHIMDLCIEC
QANQASATSEECTVAWGVCNHAFHFHCISR
WLKTRQVCPLDNREWEFQKYGH

Rbx1 (regular text): Start ***bold italics*** (amino acid residues 1-115)
Accession number: NP_055063.1

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