

Cul1/Rbx1/Skp1 [untagged]

E3 Ligase

Alternate Names: Cul1 = Cullin1, MGC149834, MGC149835
Rbx1 = HRT1, Regulator of cullins 1, Ring finger protein 75, RNF75, ROC1, ZYP protein
Skp1 = CDK2/Cyclin A associated protein p19A, Organ of corti protein 2

Cat. No. 63-1001-025

Lot. No. 30154

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 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). CRL subunits include, a scaffold protein (cullin family protein), a Ring finger protein either Rbx1 (Cul1-4) or Rbx2 (Cul5) that binds a ubiquitin-loaded E2 Ube2M or Ube2F respectively (Sarikas, *et al.*, 2011; Skowyra, *et al.*, 1997). Many CRL E3 ligases have additional linker proteins; such as Skp1 associated with

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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:

Cul1: ~89.9 kDa; Rbx1: ~12.3 kDa; Skp1: ~18.3

Purity: >95% by InstantBlue™ SDS-PAGE

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

Protein Sequences: Please see page 2

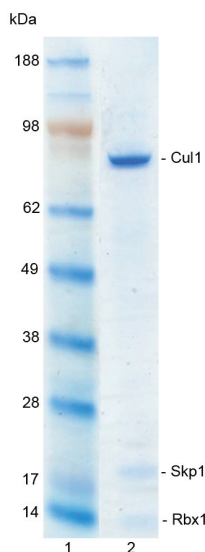
Quality Assurance

Purity:

4-12% gradient SDS-PAGE
InstantBlue™ staining

Lane 1: MW markers

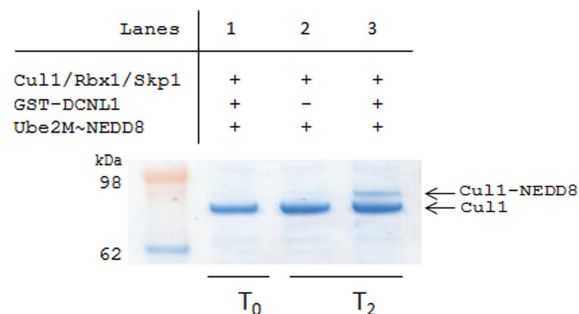
Lane 2: 1 µg Cul1/Rbx1/Skp1



Protein Identification:

Confirmed by mass spectrometry.

E3 Ligase Assay: The activity of Cul1/Rbx1/Skp1 was validated indirectly through its ability to act as a substrate for neddylation in the presence of the NEDD8 E3 ligase GST-DCNL1 and thioester-loaded His-Ube2M~NEDD8. Incubation of Cul1/Rbx1/Skp1 and thioester loaded His-Ube2M~NEDD8 in the presence or absence of GST-DCNL1 at 4°C was compared at two time points T₀ and T₂ minutes. Increased neddylation of the Cul1 subunit in the presence of GST-DCNL1 was demonstrated



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Lot-specific COA version tracker: v1.0.0

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CERTIFICATE OF ANALYSIS Page 2 of 2

Background

Continued from page 1

Cul1 and DDB1 associated with Cul4. The first CRL E3 ligase identified was named Skp1/Cullin or Cdc53/F-box (SCF) from *Saccharomyces cerevisiae*. CRLs function through their cognate substrate-recognition molecules, such as the F-box proteins SOCS, BTB and DCAF; each of these contain a distinct motif that is recognized by an adaptor molecule which is itself linked to a cognate cullin. There are approximately 61 human F-box proteins all of which can bind to Skp1 through the F-box domain. It is thought most of the F-box proteins can be assembled into the SCF E3 complex through Skp1, which binds to CUL1 (Sarikas *et al.*, 2011).

References:

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 DCN13 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.

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

Physical Characteristics

Continued from page 1

Protein Sequence: Cullin 1

GGSMSSTRSQNP HGLKQIGLDQIWDDL
RAGIQQVYTRQSMASRYMELYTHVYNYCTS
VHQSNOARGAGVPPSKSKKQTPGGAQFVGLE
LYKRLKEFLKNYLTNLLKDGEDLMDESVLK
FYTQQWEDYRFSSKVLNGICAYLNRHWVRR
DEGRKGIYEIYSLALVTWRDCLFRPLNKQVT
NAVLLKIEKERNGETINTRLISGVVQSYVEL
GLNEDDAFAKGP TLT VYKESFESQFLADTER
FYTRESTEF LQQNPVTEYMKKAEARLLEEQR
RVQVYLHES TQDELARKCEQVLI EKHLE
IFHTEFQNL DADKNE DLGRMYNLVSR IQDG
LGELKLLLETHIHNOGLAAIEKCGEAA LNDPK
MYVQTVLDVHKKNALVMSAFNNDAGFVAALD
KACGRFINNNAVTKMAQSSSKSPELLARYCD
SLLKSSKNPEEALED TLNQVMVVFYI ED
KDFVQKFYAKMLAKRLVHQNSASDDAEAS
MISKLKQACGFEYTSKLQRMFQDIGVSKDL
NEQFKKHLTNSEPLDLDFSIQVLSG SWP
FQQSCTFALPSELERSYQRF TAFYASRHS
GRKLTWLYQLSKGELVTNCFKNRYTLQASTFQ
MALLQYNTEDAYTVQQLTDSTQIKMDILAQV
LQILLKSKLLVLEDENANVDEVELKPD TLIK
LYLGYKNNKLRVNIINVP MKTEQKQE QETH
KNIEEDRKL LIQAAIVRIMKMRKVLKHQQLL
GEVLTQLSSRFKPRVPVIKCCIDILIEKEY
LERVDGEKDTYSYLA

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

Cullin1 (regular text): Start ***bold italics*** (amino acid residues 1-776)

Accession number: NP_003583.2

Cullin1 [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
NAVALWAWD IVDNCAICRNHIMDL CIEC
QANQASATSEECTVAWGVCNHAHFHCISR
WLKTRQVCP LDNREWEFQKYGH

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

Accession number: NP_055063.1

Protein Sequence: Skp1

MP SIKLQSSDGEIFEVDVEIAKQSVTIK
TMLEDLGMDDDEGDDDPVPLPNVNAAILK
KVIQWCTHHKDDPPPPEDDENKEKRTD
DIPVWDQEF LKVDQGT L FELILAANYLDIK
GLLDVTC KTVANMIK GKTP EEIRKTF
NIKNDFT EEEEEAQV GSTQFCL

Skp1 (regular text): Start ***bold italics*** (amino acid residues 1-160)

Accession number: NP_008861.2



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