Cul5/Rnf7 [untagged]

E3 Ligase

Alternate Names: Cul5 = VACM 1, Vasopressin activated calcium mobilizing receptor
Rnf7 = Regulator of Cullins 2; ROC2, Rbx2, Sensitive to Apoptosis Gene; SAG

Cat. No. **63-1002-025**
Lot. No. **30165**

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). Identification of 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). Cul-5 has been shown to form a complex with the Ring

Continued on page 2

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:
Cul5: ~91.1 kDa; Rnf7: ~12.7 kDa

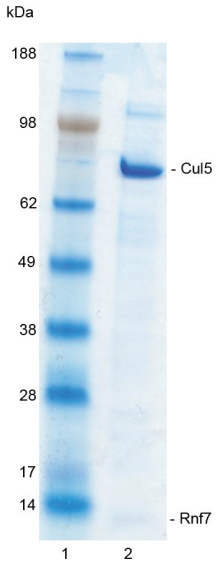
Purity: >85% by InstantBlue™ SDS-PAGE

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

Protein Sequences: Please see page 2

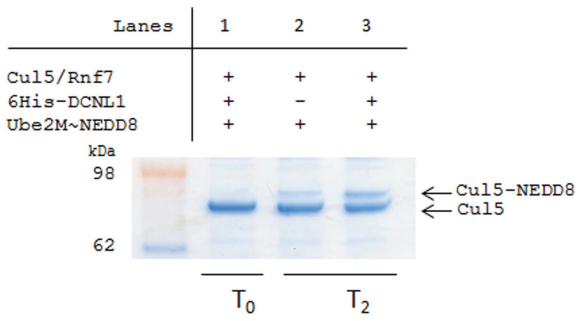
Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg Cul5/Rnf7



Protein Identification:
Confirmed by mass spectrometry.

E3 Ligase Assay: The activity of Cul5/Rnf7 was validated indirectly through its ability to act as a substrate for neddylation in the presence of the NEDD8 E3 ligase His-DCNL1 and thioester loaded His-Ube2M~NEDD8. Incubation of Cul5/Rnf7 and thioester loaded His-Ube2M~NEDD8 in the presence or absence of His-DCNL1 at 4˚C was compared at two time points T_0 and T_2 minutes. Increased neddylation of the Cul5 subunit in the presence of His-DCNL1 was demonstrated.



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Lot-specific COA version tracker: v1.0.0

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Background

Continued from page 1

finger protein Rbx2(Rnf7), the adaptor proteins Elongin B, Elongin C, and the SOCS(suppressors of cytokine signaling) box proteins to form an active CRL-5 E3 ligase (Okumura *et al.*, 2012; Sarikas *et al.*, 2011). Cul-5 also interacts with HSP90 and ErbB2. Cul-5 ubiquitylates ErbB2 – leading to its degradation – in the absence of the traditional adaptors Elongin B/C demonstrating the Elongin B/C independent E3 ligase activity of Cul-5/Rbx2 (Ehrlich *et al.*, 2009).

References:

Ehrlich ES, Wang T, Luo K, Xiao Z, Niewiadomska AM, Martinez T, Xu W, Neckers L, Yu XF. (2009) Regulation of Hsp90 client proteins by a Cullin5-RING E3 ubiquitin ligase, *PNAS* **106**, 20330-20335.

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.

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, *PNAS* **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.

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

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

Zhou W, Wei, W. and Sun, Y (2013) Genetically engineered mouse models for functional studies of SKP1-CUL1-F-box-protein (SCF) E3 ubiquitin ligases, *Cell Res* **23**, 599-619.

Physical Characteristics

Continued from page 1

Protein Sequence: Cullin 5

GGS***M***ATSNLLKNKGSLQFEDKWDFMRPIVLKLL
RQESVTKQQWFDLFSDVHAVCLWDDKGPAKI
HQALKEDILEFIKQAQARVLSHQDDTALLKAY
IVEWRKFFTQCDILPKPFCQLEITLMGKQG
SNKKSNVEDSIVRKLMLDTWNESIFSNIKN
RLQDSAMKLVHAERLGEAFDSQLVIGVRESYVN
LCSNPEDKLQIYRDNFEKAYLDSTERFYRTQA
PSYLQQNGVQNYMKYADAKLKEEEKRAL
RYLETRRECNSVEALMECCVNALVTSFKETI
LAECQGMIKRNETEKLHLMFSLMDKVPNGI
EPMLKDLEEHIISAGLADMVAAAETITTD
SEKYVEQLLTLFNRFSKLVKEAFQDDPRFL
TARDKAYKAVVNDATIFKLELPLKQKGVGLK
TQPESKCPELLANYCDMLLRKTPLSKKLT
SEEIEAKLKEVLLVLKYVQNKDVFMRYH
KAHLTRRLILDISADSEIEENMVEWLREVG
MPADYVNKLARMFQDIKVSEDLNQAFKEMH
KNNKLALPADSVNIKILNAGAWSRSSEKVFVS
LPTELEDLIPEVEEFYKKNHSGRKLHWHHLM
SNGIITFKNEVGQYDLEVTTFQLAVLFAWN
QRPREKISFENLKLATELPDAELRRTLWS
LVAFPKLKRQVLLYEPQVNSPKDFTEGTLFS
VNQEFSLIKNAKVQKRGKINLIGRLQLTTER
MREEENEGIVQLRILRTQEAIIQIMKMRK
KISNAQLQTELVEILKNMFLPQKKMIKEQ
IEWLIEHKYIRRDESDINTFIYMA

The residues underlined remain after cleavage and removal of the purification tag.
Cullin 5 (regular text): Start ***bold italics*** (amino acid residues 1-780)
Accession number: NP_003469.2

Cullin5 [Dac tagged] /Rnf7 was cleaved with TEV protease [6His tagged] (to remove the Dac tag). The Dac tag and TEV protease [6His-tagged] were removed by capturing on amp sepharose and nickel resin respectively.

Protein Sequence: Rnf7

MADVEDGEETCALASHSGSSGSKSGGDKMF
SLKKWNAVAMWSWDVECDTCAICRVQVM
DACLRCQAENKQEDCVVVWGECNHSFHNCC
MSLWVKQNNRCPLCQQDWVVQRIGK

Rnf7 (regular text): Start ***bold italics*** (amino acid residues 1-113)
Accession number: NP_055060.1

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