

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 sub-family 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

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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:

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

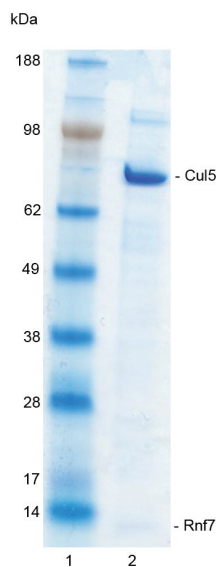
Purity:

4-12% gradient SDS-PAGE

InstantBlue™ staining

Lane 1: MW markers

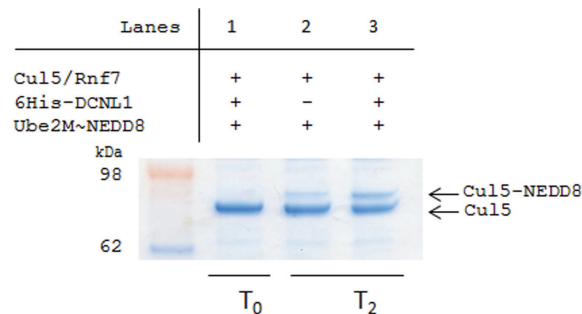
Lane 2: 1 µg Cul5/Rnf7



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 T₀ and T₂ 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

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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 DCN13 promotes Cul3 neddylation at membranes, *PNAS* **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, Nakatsukasaka K, 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

GGSMATSNNLLKNKGS~~LQ~~FEDKWFMRP I V L K L L
RQESVTKQQWFDLFS DVHAVCLWDDKGP A K I
HQALKEDI L E F I K Q A Q A R V L S H Q D D T A L L K A Y
I V E W R K F F T Q C D I L P K P F C Q L E I T L M G K Q G
S N K K S N V E D S I V R K L M L D T W N E S I F S N I K N
R L Q D S A M K L V H A E R L G E A F D S Q L V I G V R E S Y V N
L C S N P E D K L Q I Y R D N F E K A Y L D S T E R F Y R T Q A
P S Y L Q Q N G V Q N Y M K Y A D A K L K E E E K R A L
R Y L E T R R E C N S V E A L M E C C V N A L V T S F K E T I
L A E C Q G M I K R N E T E K L H L M F S L M D K V P N G I
E P M L K D L E E H I I S A G L A D M V A A A E T I T T D
S E K Y V E Q L L T L F N R F S K L V K E A F Q D D P R F L
T A R D K A Y K A V V N D A T I F K L E L P L K Q K G V G L K
T Q P E S K C P E L L A N Y C D M L L R K T P L S K K L T
S E E I E A K L K E V L L V L K Y V Q N K D V F M R Y H
K A H L T R R L I L D I S A D S E I E E N M V E W L R E V G
M P A D Y V N K L A R M F Q D I K V S E D L N Q A F K E M H
K N N K L A L P A D S V N I K I L N A G A W S R S S E K V F V S
L P T E L E D L I P E V E E F Y K K N H S G R K L H W H H L M
S N G I I T F K N E V G Q Y D L E V T T F Q L A V L F A W N
Q R P R E K I S F E N L K L A T E L P D A E L R R T L W S
L V A F P K L K R Q V L L Y E P Q V N S P K D F T E G T L F S
V N Q E F S L I K N A K V Q K R G K I N L I G R L Q L T T E R
M R E E N E G I V Q L R I L R T Q E A I I Q I M K M R K
K I S N A Q L O T E L V E I L K N M F L P Q K K M I K E Q
I E W L I E H K Y I R R D E S D I N T F I Y M A

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
SLKKNNAVAMWSWDVECDTCAICRVQVM
DACLRCAENKQEDCVVVWGECNHSFHNC
MSLWVKQNNRCPQCQDQWVVRIGK

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

Accession number: NP_055060.1



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