

DCNL1 [6His-tagged]

E3 Ligase

Alternate Names: RP42 homolog, RP42, SCRO, DCUN1D1

Cat. No. 63-2000-025

Lot. No. 30157

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 NEDDylation pathway play a pivotal role in the activation of the largest class of ubiquitin E3 ligases called Cullin-RING-Ligases (CRLs). Akin to ubiquitylation three classes of enzymes are involved in the process of mammalian NEDDylation; E1 activating enzyme (APP-BP1/UBA3 heterodimer), E2 conjugating enzymes (UBE2M or UBE2F) and E3 ligases the defective in Cul neddylation 1 domain-containing proteins (DCUN1D1-5) (Meyer-Schaller *et al.*, 2009; Huang *et al.*, 2011). There are 5 human DCUN1D1-5 proteins are also named defective in Cul neddylation 1 like proteins (DCNL1-5) (Meyer-Schaller *et al.*, 2009). Cloning of DCNL1 was first described by Kurz *et al.* (2005). The DCNLs have distinct amino-terminal domains, but share a conserved C-terminal potentiating neddylation (PONY) domain (Kurz *et al.*, 2008). DCNL1 is required for Cul1 NEDDylation in *saccharomyces cerevisiae* and *caenorhabditis elegans*. Overexpression of DCNL1 in yeast results in accumulation of NEDD8 bound to the yeast Cul1 ortholog Cdc53. N-terminal acetylation of UBE2M influences the E3 dependent ligation of NEDD8 to Cul1, which is thought to occur by the burial of the N-acetyl-methionine of UBE2M into a hydrophobic pocket in the E3 DCNL1 promoting Cul1 NEDDylation (Kurz *et al.*, 2005; Scott *et al.*, 2011).

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Physical Characteristics

Species: human

Source: *E. coli*

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: ~32.5 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

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

Protein Sequence:

M G S S H H H H H S S G L E V L F Q G
P G S M N K L K S S Q K D K V R Q F M I F T Q S S E K
T A V S C L S Q N D W K L D V A T D N F F Q N P E
L Y I R E S V K G S L D R K K L E Q L Y N R Y K D P Q D E N
K I G I D G I Q Q F C D D L A L D P A S I S V L I I A W K
F R A A T Q C E F S K Q E F M D G M T E L G C D S I E K L K A Q
I P K M E Q E L K E P G R F K D F Y Q F T F N F A K N P G Q K
G L D L E M A I A Y W N L V L N G R F K F L D L W N K F L L E
H H K R S I P K D T W N L L L D F S T M I A D D M S N Y D E E
G A W P V L I D D F V E F A R P Q I A G T K S T T V

Tag (**bold text**): N-terminal His

Protease cleavage site: PreScission™ (LEVLQ▼GP)

DCNL1 (regular text): Start **bold italics** (amino acid residues 1-259)

Accession number: NP_065691.2

Quality Assurance

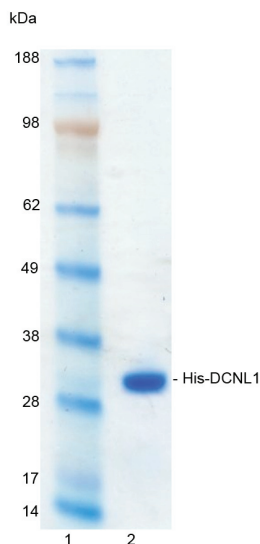
Purity:

4-12% gradient SDS-PAGE

InstantBlue™ staining

Lane 1: MW markers

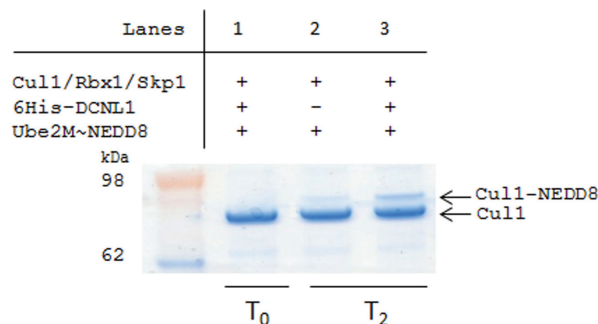
Lane 2: 1 µg His-DCNL1



Protein Identification:

Confirmed by mass spectrometry.

E3 Ligase Assay: The activity of His-DCNL1 was validated through its ability to enhance the neddylation of Cul1/Rbx1/Skp1 acting as a substrate in the presence of the thioester-loaded His-Ube2M~NEDD8. Incubation of Cul1/Rbx1/Skp1 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 Cul1 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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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.

Kurz T, Chou YC, Willems AR, Meyer-Schaller N, Hecht ML, Tyers M, Peter M, Sicheri F. (2008) Dcn1 functions as a scaffold-type E3 ligase for cullin neddylation, *Mol Cell* **29**, 23-35.

Kurz T, Ozlü N, Rudolf F, O'Rourke SM, Luke B, Hofmann K, Hyman AA, Bowerman B, Peter M. (2005) The conserved protein DCN-1/Dcn1p is required for cullin neddylation in *C. elegans* and *S. cerevisiae*, *Nature* **435**, 1257-1261.

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.

Scott, D.C, Monda JK, Bennett EJ, Harper JW, Schulman BA. (2011) N-terminal acetylation acts as an avidity enhancer within an interconnected multiprotein complex, *Science* **334**, 674-678.



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