DCNL2 [GST-tagged]

E3 Ligase

Alternate Names: C13orf17 protein, FLJ10704, FLJ20092, DCUN1D2

Cat. No.	**63-2002-025**	Quantity:	25 µg
Lot. No.	**30159**	Storage:	-70˚C

FOR RESEARCH USE ONLY NOT FOR USE IN HUMANS

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 DCNL2 was first described by Kurz *et al.* (2005) and Lamesch *et al.* (2007). The DCNLs have distinct amino-terminal domains, but share a conserved C-terminal potentiating neddylation (PONY) domain (Kurz *et al.*, 2008). It has been determined that the interaction between the DCNLs and Cul1 occurs through the PONY domain and the Winged Helix DNA binding domain (WHB) respectively (Kurz *et al.*, 2008; Scott *et al.*, 2011). Pairwise analysis of 30 combinations of the five DCNL PONY domains and six cullin WHB subdomains by isothermal titration calorimetry have all shown interaction albeit with differing affinities (Monda *et al.*, 2013).

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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: ~57.6 kDa

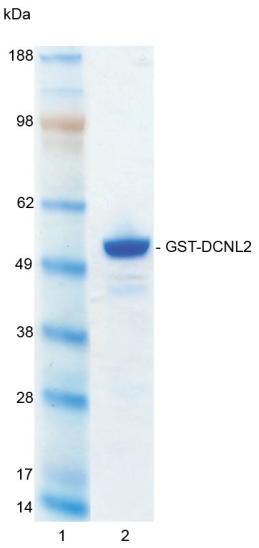
Purity: >95% by InstantBlue™ SDS-PAGE

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

Protein Sequence: Please see page 2

Quality Assurance

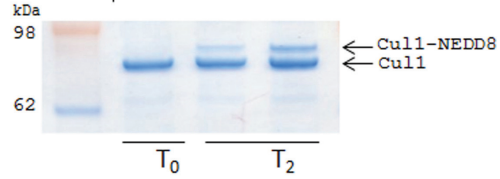
Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg GST-DCNL2



Protein Identification:
Confirmed by mass spectrometry.

E3 Ligase Assay: The activity of GST-DCNL2 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 GST-DCNL2 at 4˚C was compared at two time points T_0 and T_2 minutes. Increased neddylation of the Cul1 subunit in the presence of GST-DCNL2 was demonstrated.

Lanes	1	2	3
Cul1/Rbx1/Skp1	+	+	+
GST-DCNL2	+	–	+
Ube2M~NEDD8	+	+	+



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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.

Lamesch P, Li N, Milstein S, Fan C, Hao T, Szabo G, Hu Z, Venkatesan K, Bethel G, Martin P, Rogers J, Lawlor S, McLaren S, Dricot A, Borick H, Cusick ME, Vandenhaute J, Dunham I, Hill DE, Vidal M. (2007) hORFeome v3.1: a resource of human open reading frames representing over 10,000 human genes, *Genomics* **89**, 307-315.

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.

Monda J.K,Scott DC, Miller DJ, Lydeard J, King D, Harper JW, Bennett EJ, Schulman BA. (2013) Structural Conservation of Distinctive N-terminal Acetylation-Dependent Interactions across a Family of Mammalian NEDD8 Ligation Enzymes, *Structure* **21**, 42-53.

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

Physical Characteristics

Continued from page 1

Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYYIDGD
VKLTQSMAIIRYIADKHNMLGGCPKERAEISM
LEGAVLDIRYGVSRIAYSKDFETLKVDFL
SKLPEMLKMFEDRLCHKTYLNGDHVTHPD
FMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPQIDKYLKSSKYIAWPLQGWQATF
GGGDHPPKSDLEVLFQGPLGSPNSRVD***M***H**
KLKSSQKDKVRQFMACTQAGERTAIYCLTQNE
WRLDEATDSFFQNPDSLHRESMRNAVDKK
KLERLYGRYKDPQDENKIGVDGIQQFCD
DLSLDPASISVLVIAWKFRAATQCEFSRKE
FLDGMTELGCDSMEKLKALLPRLEQELKDTAK
FKDFYQFTFTFAKNPGQKGLDLEMAVAYWKLV
LSGRFKFLDLWNTFLMEHHKRSIPRDTWNLLL
DFGNMIADDMSNYDEEGAWPVLIDDFVEYAR
PVVTGGKRSLF

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
DCNL2 (regular text): Start ***bold italics*** (amino acid residues 1-259)
Accession number: NP_001014305.1

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Lot-specific COA version tracker: v1.0.0