

# DCNL1 [GST-tagged]

## E3 Ligase

Alternate Names: RP42 homolog, RP42, SCRO, DCUN1D1

Cat. No. 63-2001-025

Lot. No. 30158

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).

Continued on page 2

## 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: ~57.1 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

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

Protein Sequence: Please see page 2

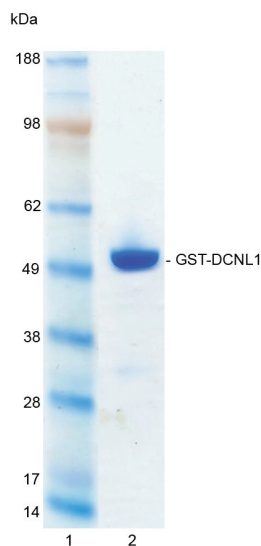
## Quality Assurance

### Purity:

4-12% gradient SDS-PAGE  
InstantBlue™ staining

Lane 1: MW markers

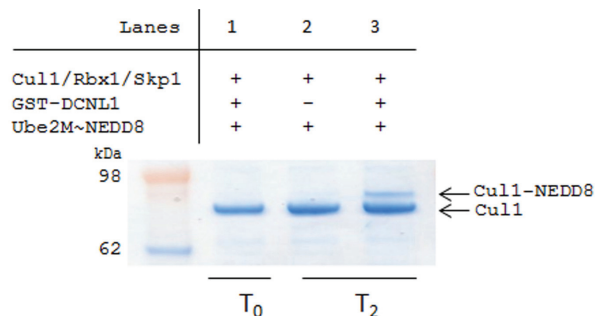
Lane 2: 1 µg GST-DCNL1



### Protein Identification:

Confirmed by mass spectrometry.

**E3 Ligase Assay:** The activity of GST-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 GST-DCNL1 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-DCNL1 was demonstrated.



www.ubiquigent.com  
Dundee, Scotland, UK

### ORDERS / SALES SUPPORT

International: +1-617-245-0020  
US Toll-Free: 1-888-4E1E2E3 (1-888-431-3233)  
Email: sales.support@ubiquigent.com

### UK HQ and TECHNICAL SUPPORT

International: +44 (0) 1382 381147 (9AM-5PM UTC)  
US/Canada: +1-617-245-0020 (9AM-5PM UTC)  
Email: tech.support@ubiquigent.com

Email services@ubiquigent.com for enquiries regarding compound profiling and/or custom assay development services.

© Ubiquigent 2013. Unless otherwise noted, Ubiquigent, Ubiquigent logo and all other trademarks are the property of Ubiquigent, Ltd.

Limited Terms of Use: For research use only. Not for use in humans or for diagnostics. Not for distribution or resale in any form, modification or derivative OR for use in providing services to a third party (e.g. screening or profiling) without the written permission of Ubiquigent, Ltd.

Lot-specific COA version tracker: v1.0.0

# DCNL1 [GST-tagged]

## E3 Ligase

Alternate Names: RP42 homolog, RP42, SCRO, DCUN1D1

Cat. No. 63-2001-025

Lot. No. 30158

Quantity: 25 µg

Storage: -70°C



FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 2 of 2

## Background

Continued from page 1

### 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 Cui3 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.

## Physical Characteristics

Continued from page 1

### Protein Sequence:

**MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH**  
**LYERDEGDKWRNKKFELGLEFPNLPYYIDGD**  
**VKLTQSMAIRYIADKHNLGGCPKERAEISM**  
**LEGAVLDIRYGVSR IAYSKDFETLKVDFL**  
**SKLPEMLKMFEDRLCHKTYLNGDHVTHPD**  
**FMLYDALDVVLYMDPMCLDAFPKLVCFK**  
**KRIEAIPOIDKYLKSSKYIAWPLQGWQATFG**  
**GGDHPKSDLEVLFGPPLGSGSMNKLKSSQKD**  
**KVRQFMIFQTQSSEKTAVSCLSQNDWKLDVATD**  
**NFFQNPELYIRESVKGSLDRKKLEQLYN**  
**RYKDPQDENKIGIDGIQQFCDDLALDPASIS**  
**VLI IAWKFRAATQCEFSKQEFMDGMTELCD**  
**SIEKLKAQIPKMEQELKEPGRFKDFYQFTFN**  
**FAKNPGQKGLDLEMAIAYWNLVLNGRFKFLDL**  
**WNKFLEHHKRSIPKDTWNLLLLDFSTMIADDM**  
**SNYDEEGAWPVLIDDFVEFARPOIAGTKSTTV**

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

Protease cleavage site: PreScission™ (LEVLFG▼GP)

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

Accession number: NP\_065691.2



www.ubiquigent.com  
Dundee, Scotland, UK

### ORDERS / SALES SUPPORT

International: +1-617-245-0020  
US Toll-Free: 1-888-4E1E2E3 (1-888-431-3233)  
Email: sales.support@ubiquigent.com

### UK HQ and TECHNICAL SUPPORT

International: +44 (0) 1382 381147 (9AM-5PM UTC)  
US/Canada: +1-617-245-0020 (9AM-5PM UTC)  
Email: tech.support@ubiquigent.com

Email services@ubiquigent.com for enquiries regarding compound profiling and/or custom assay development services.

© Ubiquigent 2013. Unless otherwise noted, Ubiquigent, Ubiquigent logo and all other trademarks are the property of Ubiquigent, Ltd.

Limited Terms of Use: For research use only. Not for use in humans or for diagnostics. Not for distribution or resale in any form, modification or derivative OR for use in providing services to a third party (e.g. screening or profiling) without the written permission of Ubiquigent, Ltd.

Lot-specific COA version tracker: v1.0.0