

DCNL4 (human; full length), pAb

Alternate Name: DCUN1D4

Cat. No. 68-0008-100
Lot. No. 30245

Quantity: 100 µg
Storage: -20°C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS

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This antibody was developed and validated by the Medical Research Council Protein Phosphorylation and Ubiquitylation Unit (University of Dundee, Dundee, UK).

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 the E3 ligases defective in Cul NEDDylation 1 domain-containing proteins (DCUN1D1-5) (Meyer-Schaller *et al.*, 2009; Huang *et al.*, 2011). The 5 human DCUN1D1-5 proteins are also named defective in Cul NEDDylation 1 like proteins (DCNL1-5) (Meyer-Schaller *et al.*, 2009). Cloning of DCNL4 was first described by 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

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: human DCNL4 (residues 1 – 119) [GST-tagged]

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

Specificity: detects DCNL4 at ~34 kDa

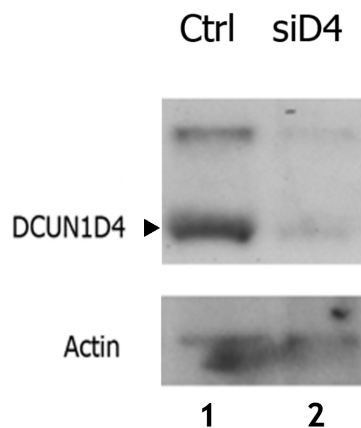
Reactivity: human; other species not tested

Stability/Storage: 12 months at -20°C; aliquot as required

Research Applications and Quality Assurance

Western Immunoblotting:
Use 1.0 µg/ml

Immunoprecipitation:
Use 2.0 µg/mg of cell extract



Western Blotting Analysis:

U2OS cells were transfected with either control siRNA (Ctrl) or DCNL4 siRNA (siD4) (lanes 1 and 2). By Western blotting the specific recognition of a band corresponding to DCNL4 (DCUN1D4) was observed in lysates treated with control siRNA (lane 1) compared to lysates treated with DCNL4 siRNA (lane 2) where the presence of DCNL4 could not be detected when probed with 1.0 µg/ml anti-DCNL4 antibody (Cat# 68-0008-100). The upper band in the DCNL4 blot is thought to be DCNL4 modified by a UBL (e.g. Ubiquitin or SUMO) hence why it is also decreased upon siD4 treatment.



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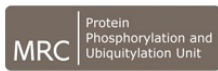
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Lot-specific COA version tracker: v1.0.0



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Background

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Antibody Production:

Anti-DCNL4 (human) polyclonal antibody was raised in sheep against DCNL4 (residues 1-119 of human DCNL4). The antibodies were purified by the Medical Research Council Protein Phosphorylation and Ubiquitylation Unit (MRC-PPU, University of Dundee, Dundee, U.K.) by affinity purification of the anti-DCNL4 pAbs from the sheep serum using an antigen-agarose column followed by depletion of any anti-GST pAbs using a GST-agarose column. Anti-DCNL4 (human) pAb was sourced by Ubiquigent directly from the MRC-PPU.

General References:

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.

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.

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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.

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.



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