



DCNL3 (human; full length), pAb

Alternate Names: DCUN1D3, FLJ41725, MGC48972

Cat. No. 68-0007-100
Lot. No. 30244

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 DCNL3 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 interactions, albeit with differing affinities (Monda *et al.*, 2013). DCNL3 has a conserved lipid-

Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: human DCNL3 (residues 1 – 105) [GST-tagged]

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

Specificity: detects DCNL3 at ~34 kDa

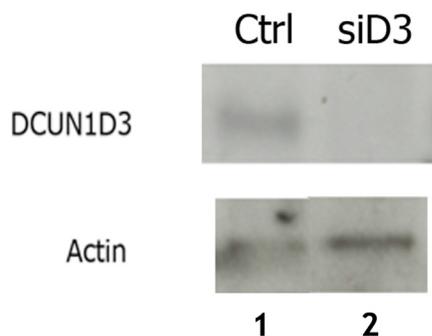
Reactivity: human; other species not tested

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 DCNL3 siRNA (siD3) (lanes 1 and 2). By Western blotting the specific recognition of a band corresponding to DCNL3 (DCUN1D3) was observed in lysates treated with control siRNA (lane 1) compared to lysates treated with DCNL3 siRNA where the presence of DCNL3 could not be detected when probed with 1.0 µg/ml anti-DCNL3 antibody (Cat# 68-0007-100).

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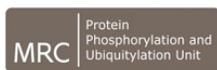
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Lot-specific COA version tracker: v1.0.0



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Background

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modified motif in the N terminal region of the protein, and membrane-bound DCNL3 is able to recruit Cullin3 to membranes and is functionally important for the NEDDylation Cullin3 *in vivo* (Meyer-Schaller *et al.*, 2009).

Antibody Production:

Anti-DCNL3 (human) polyclonal antibody was raised in sheep against DCNL3 (residues 1-105 of human DCNL3). 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-DCNL3 pAbs from the sheep serum using an antigen-agarose column followed by depletion of any anti-GST pAbs using a GST-agarose column. Anti-DCNL3 (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.

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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.

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