





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 E3 ligases the defective in Cul NEDDvlation 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 DCNL5 was first described by Lamesch et al. (2007). The DCNLs have distinct aminoterminal domains, but share a conserved Cterminal 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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# DCNL5 (mouse; full length), pAb

Alternate Names: DCUN1D5, FLJ32431, FLJ37425, MGC2714

**Cat. No. 68-0009-100** Quantity: 100 μg **Lot. No. 30246** Storage: -20°C

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# **Physical Characteristics**

Quantity: 100 µg

Concentration: to be provided on

shipping

Source: sheep polyclonal antibody

Immunogen: mouse DCNL5 (residues

1 – 237) [GST-tagged]

Purification: affinity-purified using

immobilized immunogen

Formulation: phosphate-buffered

saline

Specificity: detects DCNL5 at

~28 kDa

Reactivity: mouse; other species not

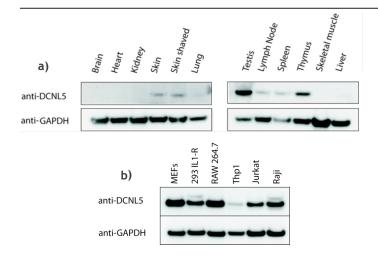
tested

Stability/Storage: 12 months at

-20°C; aliquot as required

### **Research Applications and Quality Assurance**

Western Immunoblotting: Use 1.0 µg/ml Immunoprecipitation: Use 5.0 µg/mg of cell extract



### Western Blotting Analysis:

By western blotting the specific recognition of DCNL5 could be observed in several a) mouse tissues and b) cell lines when probed with 1.0  $\mu$ g/ml of anti-mouse DCNL5 antibody (Cat# 68-0009-100).

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Lot-specific COA version tracker: v1.0.0







# DUNDEE

### **Background**

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### **Antibody Production:**

Anti-DCNL5 (mouse) polyclonal antibody was raised in sheep against DCNL5 (residues 1-237 of mouse DCNL5). 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-DCNL5 pAbs from the sheep serum using an antigenagarose column followed by depletion of any anti-GST pAbs using a GST-agarose column. Anti-DCNL5 (mouse) 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,

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) hOR-Feome 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 I G, Sicheri F, Peter M (2009) The human Don1. 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) Nterminal acetylation acts as an avidity enhancer within an interconnected multiprotein complex, Science 334, 674-678.

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