DCNL5 (human; full length), pAb

Alternate Names: DCUN1D5, FLJ32431, FLJ37425, MGC2714

Cat. No.	**68-0010-100**	Quantity:	100 µg
Lot. No.	**30247**	Storage:	-20˚C

FOR RESEARCH USE ONLY | NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS | **Page 1 of 2**

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 DCNL5 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).

Continued on page 2

Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: human DCNL5 (residues 1 – 124)

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

Specificity: detects DCNL5 at ~28 kDa

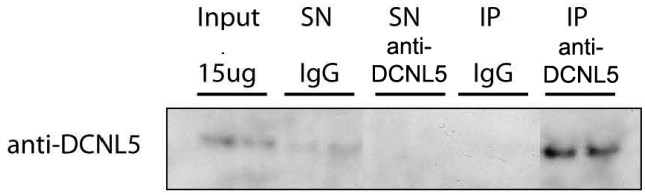
Reactivity: human; other species not tested

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

Research Applications and Quality Assurance

Western Immunoblotting:
Use 1.0 µg/ml

Immunoprecipitation:
Use 5.0 µg/mg of cell extract



Western Blotting Analysis:
DCNL5 was immunoprecipitated from total cell extracts (1mg) using 5.0 µg of anti-DCNL5 antibody (Cat# 68-0010-100). By Western Blotting DCNL5 was detected using anti-DCNL5 antibody (Cat# 68-0010-100). To demonstrate that all DCNL5 was immunoprecipitated from the input cell extract, DCNL5 could not be detected in the supernatant (SN), therefore 5.0 µg of DCNL5 antibody is sufficient to deplete DCNL5 from 1 mg of cell extract.

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.

Lot-specific COA version tracker: v1.0.0

DCNL5 (human; full length), pAb

Alternate Names: DCUN1D5, FLJ32431, FLJ37425, MGC2714

Cat. No.	**68-0010-100**	Quantity:	100 µg
Lot. No.	**30247**	Storage:	-20˚C

FOR RESEARCH USE ONLY NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS

Background

Continued from page 1

Antibody Production:
Anti-DCNL5 (human) polyclonal antibody was raised in sheep against DCNL5 (residues 1-124 of human 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 a GST-tagged antigen-agarose column. Anti-DCNL5 (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.

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. *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.

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.

Lot-specific COA version tracker: v1.0.0