

Cullin5 (human; residues 577 - 689), pAb

Alternate Names: VACM 1, Vasopressin activated calcium mobilizing receptor

Cat. No. 68-0005-100
Lot. No. 30242

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 ubiquitylation pathway play a pivotal role in a number of cellular processes including the regulated and targeted proteasome dependent degradation of substrate proteins. Three classes of enzymes are involved in the process of ubiquitylation; activating enzymes (E1s), conjugating enzymes (E2s) and protein ligases (E3s). Cullin-RING-Ligases (CRLs) are one largest class of ubiquitin E3 ligases and the enzymes of the NEDDylation pathway play a pivotal role in the activation of these, akin to ubiquitylation, the E1 activating enzyme (APP-BP1/UBA3 heterodimer) and the E2 conjugating enzymes (UBE2M or UBE2F) are involved in mammalian NEDDylation of the Cullin Ring Ligases (CRLs) (Meyer-Schaller *et al.*, 2009; Huang *et al.*, 2011; Morimoto *et al.*, 2003). Identification of the human Cullin1-5 genes were first described by Kipreos *et al.* (1996). Cullin RING ligases (CRL) comprise the largest subfamily of ubiquitin ligases and which are activated by NEDDylation. CRLs are involved in cell cycle regulation, DNA replication, DNA damage response (DDR). CRLs comprise subunits including, a scaffold protein (cullin family protein), a Ring finger protein either Rbx1 (Cul1-4) or Rbx2 (Cul5) that binds a ubiquitin E2 UBE2M or UBE2F respectively (Sarikas *et al.*, 2011; Skowyra *et al.*, 1997). Cul-5 has been shown to form a complex with the Ring finger protein Rbx2 (Rnf7), the adaptor proteins Elongin B, Elongin C, and the SOCS (suppressors of cytokine signalling) box proteins to form an active CRL-5 E3 ligase (Okumura *et al.*, 2012; Sarikas *et al.*, 2011). Cul-5 also interacts with HSP90 and ErbB2. Cul-5 ubiquitylates ErbB2 – lead-

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Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: human Cullin5 (residues 577-689) [GST-tagged]

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

Specificity: detects Cullin5 at ~91 kDa

Reactivity: human

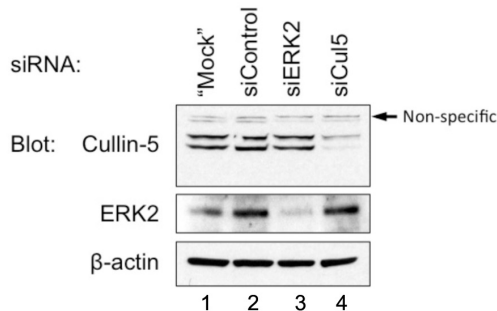
Species cross reactivity: mouse, chicken

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

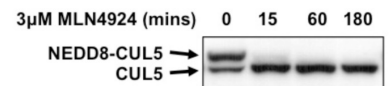
Research Applications and Quality Assurance

Western Immunoblotting:
Use 0.1-0.5 µg/ml

Immunoprecipitation:
Use 3.0 µg/mg of cell extract



Panel A



Panel B

Western Blotting Analysis:

A) U2OS cells were “Mock” transfected or transfected with either control siRNA, siERK2 or siCul5 (lanes 2, 3 and 4). Cell lysates were denatured in SDS and subjected to SDS-PAGE. Western blotting was carried out with 0.5 µg/ml anti-Cullin5 antibody (Cat# 68-0005-100). A doublet was detected in those lysates from cells treated with “Mock”, siControl and siERK2 (the upper of the two bands corresponding to the NEDD8-conjugated form of Cullin5), compared to lysates from cells treated with siCul5 where Cullin5 could not be detected.

B) By Western blotting using the anti-Cullin5 antibody (Cat# 68-0005-100), the upper band of the doublet was confirmed as being NEDD8-conjugated Cullin5, through an inhibition timecourse of NEDDylation using the compound MLN4924 (an APP-BP1/UBA3 inhibitor). Following treatment of U2OS cells for 15 minutes with MLN4924 the NEDDylated form of Cullin5 could not be detected.

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Dundee, Scotland, UK

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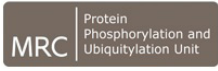
International: +44 (0) 1382 381147 (9AM-5PM UTC)
US/Canada: +1-617-245-0020 (9AM-5PM UTC)
Email: tech.support@ubiquigent.com

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

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Lot-specific COA version tracker: v1.0.0



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Background

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ing to its degradation – in the absence of the traditional adaptors Elongin B/C demonstrating the Elongin B/C independent E3 ligase activity of Cul-5/Rbx2 (Ehrlich *et al.*, 2009). Two members of the Ariadne subfamily of Ring Between Ring (RBR) ligases, Triad Domain-Containing Protein 1 (TRIAD1) and Human Homologue of *Drosophila Ariadne* (HHARI), have been shown to associate with NEDDylated CRL complexes, resulting in a stimulation of their activity (Kelsall *et al.*, 2013).

Antibody Production:

Anti-Cullin5 (human) polyclonal antibody was raised in sheep against Cullin5 (residues 577-689 of human Cullin5). 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-Cullin5 pAbs from the sheep serum using an antigen-agarose column followed by depletion of any anti-GST pAbs using a GST-agarose column. Anti-Cullin5 (human) pAb was sourced by Ubiquigent directly from the MRC-PPU.

General References:

Ehrlich ES, Wang T, Luo K, Xiao Z, Niewiadomska AM, Martinez T, Xu W, Neckers L, Yu XF (2009) Regulation of Hsp90 client proteins by a Cullin5-RING E3 ubiquitin ligase. *PNAS* **106**, 20330-20335.

Huang G, Kaufman A J, Ramanathan Y, Singh B (2011) SCRO (DCUN1D1) promotes nuclear translocation and assembly of the neddylation E3 complex. *J Biol Chem* **286**, 10297-10304.

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.

Morimoto M, Nishida T, Nagayama Y, Yasuda H (2003) Neddylation of Cul1 is promoted by Roc1 as a Neddylation E3 ligase and regulates its stability. *Biochem Biophys Res Commun* **301**, 392-398.

Okumura F, Matsuzaki M, Nakatsukasa K, Kamura T (2012) The Role of Elongin BC-Containing Ubiquitin Ligases. *Front Oncol* **2**, 1-13.

Sarikas A, Hartmann T, Pan ZQ (2011) The cullin protein family. *Genome Biology* **12**, 220.

Zhou W, Wei W, and Sun, Y (2013) Genetically engineered mouse models for functional studies of SKP1-CUL1-F-box-protein (SCF) E3 ubiquitin ligases. *Cell Res* **23**, 599-619.

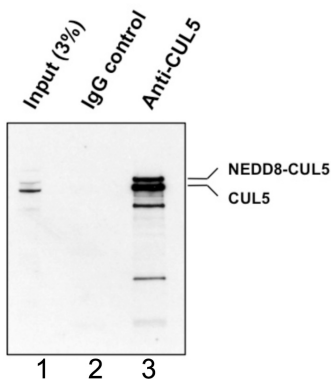
Skowyra D, Craig KL, Tyers M, Elledge SJ, Harper JW (1997) F-box proteins are receptors that recruit phosphorylated substrates to the SCF ubiquitin-ligase complex. *Cell* **9**, 1209-19.

Application Reference:

Kelsall IR, Duda DM, Olszewski JL, Hofmann K, Knebel A, Langevin F, Wood N, Wrightman M, Schulman BA, Alpi AF (2013) TRIAD1 and HHARI bind to and are activated by distinct neddylation Cullin-RING ligase complexes. *EMBO J* **32**, 2848-60.

Research Applications and Quality Assurance

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Immunoprecipitation Assay:

Cullin5 was immunoprecipitated from HEK293 total cell extracts (3 mg) using 30µg of anti-Cullin5 antibody (Cat# 68-0005-100) or pre-immune serum (IgG). Cullin5 and NEDD8 conjugated Cullin5 (lane 3) were subsequently detected by Western Blot using the same anti-Cullin5 antibody used in the immunoprecipitation.



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