

## Cullin3 (human; residues 554 - 768), pAb

Alternate Name: KIAA0617

Cat. No. 68-0004-100  
Lot. No. 30241

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 of the largest classes 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). 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). Cul3 expression in

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### Physical Characteristics

**Quantity:** 100 µg

**Concentration:** to be provided on shipping

**Source:** sheep polyclonal antibody

**Immunogen:** human Cullin3 (residues 554-768) [GST-tagged]

**Purification:** affinity-purified using immobilized immunogen

**Formulation:** phosphate-buffered saline

**Specificity:** detects Cullin3 at ~89 kDa

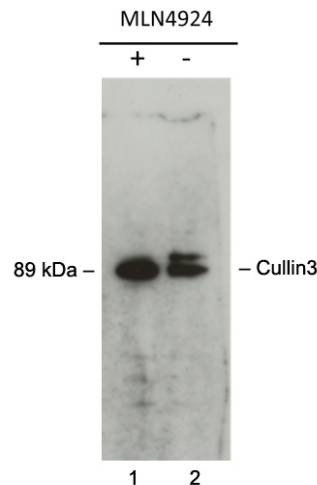
**Reactivity:** human; other species not tested

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

### Research Applications and Quality Assurance

**Western Immunoblotting:**  
Use 1.0 µg/ml

**Immunoprecipitation:**  
Not tested.



#### Western Blotting Analysis:

Whole cell U2OS extracts were treated with the compound MLN4924 which prevents cullin NEDDylation by inhibiting NAE1 (the E1 activating enzyme for NEDD8) and serves as a control to identify the unneddylated form of Cullin3. Western blotting was carried out with 1µg/ml anti-Cullin3 (Cat# 68-0004-100). In cell lysates from MLN4924 treated cells a single band was detected of the expected molecular weight corresponding to Cullin3 (lane 1), whereas in untreated cell lysates a doublet was detected, with the lower band corresponding to Cullin3 and the upper to the NEDD8-conjugated form of Cullin3 (lane 2).



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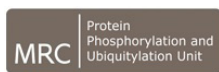
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Lot-specific COA version tracker: v1.0.0



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## Background

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human fibroblasts is induced by phorbol 12-myristate 13-acetate (PMA) and suppressed by salicylate (Du *et al.*, 1998). The Cul3/Kelch like 9 (KLHL9) / Kelch like 13 (KLHL13) complex is an E3 ligase that controls the behaviour of Aurora B on mitotic chromosomes thereby coordinating mitotic progression and completion of cytokinesis (Sumara *et al.*, 2007). Interaction of Cul3 with Kelch like 7 (KLHL7) leads to the ubiquitylation of the dopamine receptor D4 (DRD4) (Rondou *et al.*, 2008).

### Antibody Production:

Anti-Cullin3 (human) polyclonal antibody was raised in sheep against Cullin3 (residues 554-768 of human Cullin3). 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-Cullin3 pAbs from the sheep serum using an antigen-agarose column followed by depletion of any anti-GST pAbs using a GST-agarose column. Anti-Cullin3 (human) pAb was sourced by Ubiquigent directly from the MRC-PPU.

### General References:

Du M, Sansores-Garcia L, Zu Z, Wu KK, (1998) Cloning and expression analysis of a novel salicylate suppressible gene, Hs-CUL-3, a member of cullin/Cdc53 family. *J Biol Chem* 273, 24289-24292.

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.

Kipreos ET, Lander LE, Wing JP, He WW, Hedgecock EM (1996) cul-1 is required for cell cycle exit in *C. elegans* and identifies a novel gene family. *Cell* 85, 829-839.

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) Nedd8-modification of Cul1 is promoted by Roc1 as a Nedd8-E3 ligase and regulates its stability. *Biochem Biophys Res Commun* 301, 392-398.

Rondou P, Haegeman G, Vanhoenacker P, Van Craenenbroeck K (2008) BTB protein KLHL12 targets the dopamine D4 receptor for ubiquitination by a Cul3-based E3 ligase. *J Biol Chem* 283, 11083-11096.

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

Skowyra D, Craig KL, Tyers M, Elledge SJ, Harper J.W (1997) F-box proteins are receptors that recruit phosphorylated substrates to the SCF ubiquitin-ligase complex. *Cell* 91, 209-219.

Sumara I, Quadroni M, Frei C, Olma M. H, Sumara G, Ricci R, Peter MA (2007) Cul3-based E3 ligase removes Aurora B from mitotic chromosomes, regulating mitotic progression and completion of cytokinesis in human cells. *Dev Cell* 12, 887-900.



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