Cullin3 (human; residues 554 - 768), pAb

Alternate Name: KIAA0617

Cat. No.	**68-0004-100**	Quantity:	100 µg
Lot. No.	**30241**	Storage:	-20˚C

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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 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

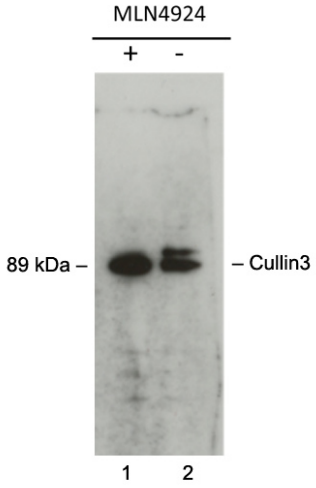
Reactivity: human; other species not tested

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 (lane2).

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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:

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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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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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