



CAND1 (human; residues 5 - 245), pAb

Alternate Names: Cullin associated nedd8 dissociated protein 1, KIAA0829, TIP120, p120 (CAND1)

Cat. No. 68-0003-100
Lot. No. 30240

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). Cullin-associated and NEDDylation dissociated (CAND1) is a cullin1 binding protein and cloning of the gene was first described by Yogosawa *et al.* (1996). CAND1 protein contains multiple HEAT repeats and is an inhibitor of several cullin-RING ubiquitin ligases (CRLs). CAND1 inhibits CRLs by binding to the cullin-RBX complexes that are unconjugated to NEDD8 and are not associated with a substrate (Liu *et al.*, 2002; Zheng *et al.*, 2002; Min *et al.*, 2003). Crystallography studies have shown that the

Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: human CAND1 (residues 5-245) [GST-tagged]

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

Specificity: detects CAND1 at ~136 kDa

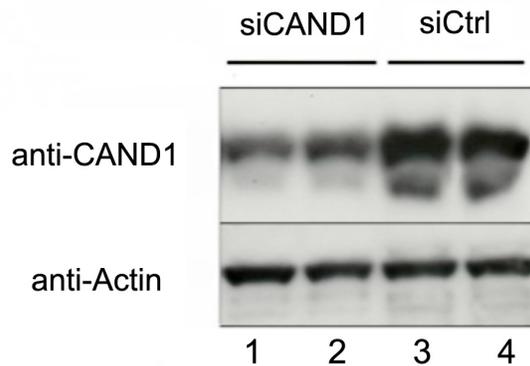
Reactivity: human; other species not tested

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

Research Applications and Quality Assurance

Western Immunoblotting:
Use 1.0 µg/ml

Immunoprecipitation:
Use 2.0 µg/mg of cell extract



Western Blotting Analysis:

Probing a Western blot with the anti-CAND1 antibody (Cat# 68-0003-100) at 1.0 µg/ml both bands of a doublet in lysates derived from cells treated with control siRNA ('siCtrl'; lanes 3 and 4) were reduced in intensity upon treatment of those cells with siRNA to CAND1 ('siCAND1'; lanes 1 and 2). The doublet being two forms of CAND1 – the properties of the variants have not been defined.

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Lot-specific COA version tracker: v1.0.0



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crystal structure of human CAND1 bound to the CUL1-RBX1 complex suggests that CAND1 inhibits CRL activity by sterically blocking both the substrate recognition subunit binding site and the NEDD8 conjugation site (Liu *et al.*, 2002; Min *et al.*, 2003; Goldenberg *et al.*, 2004). The ability of CAND1 to negatively regulate CRL assembly influences CRL activation cycles and allows the CRLs to bind distinct substrate recognition subunits which protects these complexes from undergoing ubiquitin-dependent degradation (Schmidt *et al.*, 2009; Wu *et al.*, 2006).

Antibody Production:

Anti-CAND1 (human) polyclonal antibody was raised in sheep against CAND1 (residues 5-245 of human CAND1). 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-CAND1 pAbs from the sheep serum using an antigen-agarose column followed by depletion of any anti-GST pAbs using a GST-agarose column. Anti-CAND1 (human) pAb was sourced by Ubiquigent directly from the MRC-PPU.

General References:

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Min KW, Hwang JW, Lee JS, Park Y, Tamura TA, Yoon JB (2003) TIP120A associates with cullins and modulates ubiquitin ligase activity. *J Biol Chem* **278**, 15905-10.

Schmidt MW, McQuary PR, Wee S, Hofmann K, Wolf DA (2009) F-box-directed CRL complex assembly and regulation by the CSN and CAND1. *Mol Cell* **35**, 586-97.

Yogosawa S, Makino Y, Yoshida T, Kishimoto T, Muramatsu M., Tamura T (1996) Molecular cloning of a novel 120-kDa TBP-interacting protein. *Biochem Biophys Res Commun* **229**, 612-617.

Wu JT, Chan YR, Chien CT (2006) Protection of cullin-RING E3 ligases by CSN-UBP12. *Trends Cell Biol* **16**, 362-9

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