# CYLD (human; full length), pAb

**Alternate Names:** CYLD1, KIAA0849

| | | | |
|---|---|---|---|
| **Cat. No.** | **68-0006-100** | Quantity: | 100 µg |
| **Lot. No.** | **30243** | 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 deubiquitylating enzymes (DUBs) regulate ubiquitin dependent signalling pathways. The activities of the DUBs are diverse and include the generation of free ubiquitin from precursor molecules, the recycling of ubiquitin following substrate degradation to maintain cellular ubiquitin homeostasis and the removal of ubiquitin or ubiquitin-like protein (UBL) modifications through chain editing to rescue proteins from proteasomal degradation or to influence cell signalling events (Komander *et al.*, 2009). There are two main classes of DUB, cysteine proteases and metalloproteases. CYLD is a cytoplasmic deubiquitylating enzyme belonging to the Ubiquitin Carboxy-terminal Hydrolase (UCH) family and cloning of the gene was first described by Bignell *et al.* (2000). CYLD comprises a Cytoskeletal-Associated Protein-Glycine-conserved (CAP-GLY) domain, a proline rich region, an SH3 binding domain and a sequence homology to the catalytic domain of a UCH. CYLD has been identified as a tumour suppressor protein and negatively regulates the c-Jun NH(2)-terminal kinase (JNK) signalling pathway by inhibiting the activation of Map-Kinase Kinase7 (MKK7) (Reiley *et al.*, 2004). CYLD is a negative regulator of the NF-kappaB (NFkB) signalling pathway by inhibiting the TNFR-Associated Factor 2 (TRAF2) me-

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## Physical Characteristics

**Quantity:** 100 µg

**Concentration:** to be provided on shipping

**Source:** sheep polyclonal antibody

**Immunogen:** human CYLD (residues 2-956) [His-tagged]

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

**Formulation:** phosphate-buffered saline

**Specificity:** detects CYLD at ~110 kDa

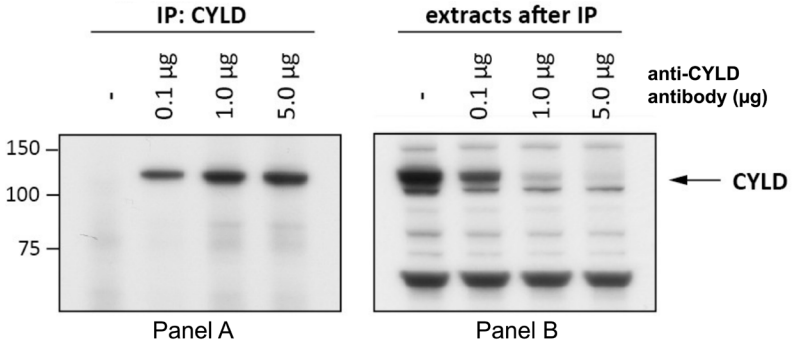
**Reactivity:** human; other species not tested

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

## Research Applications and Quality Assurance

**Western Immunoblotting:**
Use 1 µg/ml

**Immunoprecipitation:**
Use 0.7 µg/mg of cell extract




**Immunoprecipitation Assay:**
CYLD was immunoprecipitated from HEK293-IL1R total cell extracts (1.5 mg) using various amounts of anti-CYLD antibody (Cat# 68-0006-100). CYLD was subsequently detected by Western Blot (Panel A) using a commercially available anti-CYLD antibody. In order to show that all CYLD had been immunoprecipitated from the input cell extract, a Western Blot was carried out using anti-CYLD antibody (Cat# 68-0006-100) on 20 µg of the cell supernatant following immunoprecipitation - 'extracts after IP' - and only small amounts of CYLD could be detected. This demonstrates that 1 µg of the anti-CYLD antibody (Cat# 68-0006-100) can largely deplete CYLD from 1.5 mg of HEK293-IL1R total cell extract.

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**Lot-specific COA version tracker:** v1.0.0

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**CERTIFICATE OF ANALYSIS** 

## Background

*Continued from page 1*

diated activation of IKappaB Kinase (IKK) (Kovalenko *et al.*, 2003). Mutated CYLD is known to be associated with cylindromatosis, multiple familial trichoepithelioma, and Brooke-Spiegler syndrome (Hellerbrand *et al.*, 2007; Trompouki *et al.*, 2003).

**Antibody Production:**
Anti-CYLD (human) polyclonal antibody was raised in sheep against CYLD (residues 2-956 of human CYLD). 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-CYLD pAbs from the sheep serum using a 6His-tagged-antigen-agarose column. Anti-CYLD (human) pAb was sourced by Ubiquigent directly from the MRC-PPU.

**General References:**


Bignell G, Warren W, *et al.* (2000) Identification of the familial cylindromatosis tumour-suppressor gene. *Nat Genet* **25**, 160-5.

Hellerbrand C, Bumes E, Bataille F, Diemaier W, Massoumi R, Bosserhoff AK (2007) Reduced expression of CYLD in human colon and hepatocellular carcinomas. *Carcinogenesis* **28**, 21-7.

Komander D, Clague MJ, Urbe S (2009) Breaking the chains: structure and function of the deubiquitinases. *Nat Rev Mol Cell Biol* **10**, 550-63.

Kovalenko A, Chable-Bessia C, Cantarella G, Israel A, Wallach D, Courtois G (2003) The tumour suppressor CYLD negatively regulates NF-kappaB signalling by deubiquitination. *Nature* **424**, 801-5.

Reiley W, Zhang M, Sun SC (2004) Negative regulation of JNK signaling by the tumor suppressor CYLD. *J Biol Chem* **279**, 55161-7.

Trompouki E, Hatzivassiliou E, Tsichritzis T, Farmer H, Ashworth A, Mosialos G (2003) CYLD is a deubiquitinating enzyme that negatively regulates NF-kappaB activation by TNFR family members. *Nature* **424**, 793-6.



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