CYLD [6His-tagged]

Deubiquitylating Enzyme

Alternate Names: CYLD1, KIAA0849

Cat. No.	**64-0010-050**	Quantity:	50 µg
Lot. No.	**1743**	Storage:	-70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

Background

The deubiquitylating enzymes (DUBs) regulate ubiquitin dependent signaling 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 (NFκB) signalling pathway by inhibiting the TNFR-Associated Factor 2 (TRAF2) mediated activation of IKappaB Kinase (IKK) (Kovalenko *et al*.,

Continued on page 2

Physical Characteristics

Species: human

Source: Sf21 insect cell-baculovirus expression

Quantity: 50 µg

Concentration: 0.5 mg/ml

Formulation: 50 mM HEPES pH 7.5, 150 mM sodium chloride, 2 mM dithiothreitol, 10% glycerol

Molecular Weight: ~110 kDa

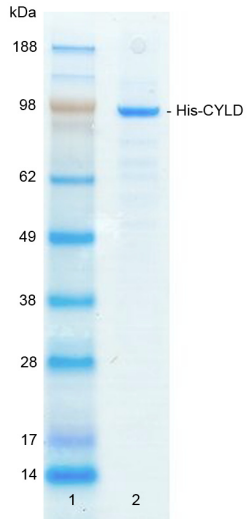
Purity: >75% by InstantBlue™ SDS-PAGE

Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequence: Please see page 2

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg His-CYLD



Protein Identification:
Confirmed by mass spectrometry.

Deubiquitylating Enzyme Assay:
The activity of His-CYLD was validated by determining the increase in fluorescence measured as a result of the enzyme catalysed cleavage of the fluorogenic substrate Ubiquitin-Rhodamine110-Glycine generating Ubiquitin and Rhodamine110-Glycine. Incubation of the substrate in the presence or absence of His-CYLD was compared confirming the deubiquitylating activity of His-CYLD.

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Lot-specific COA version tracker: v1.0.0

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Background

Continued from page 1

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).

References:

Bignell GR, 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.

Physical Characteristics

Continued from page 1

Protein Sequence:

M S Y Y H H H H H H D Y D I P T T E N L Y
F Q G A M G S ***S*** S G L W S Q E K V T S P Y W E E R I
F Y L L L Q E C S V T D K Q T Q K L L K V P K G
S I G Q Y I Q D R S V G H S R I P S A K G K K N Q
I G L K I L E Q P H A V L F V D E K D V V E I N E K F
T E L L L A I T N C E E R F S L F K N R N R L S K G L Q
I D V G C P V K V Q L R S G E E K F P G V V R F R G P L
L A E R T V S G I F F G V E L L E E G R G Q G F T D G V Y
Q G K Q L F Q C D E D C G V F V A L D K L E L I E D D D
T A L E S D Y A G P G D T M Q V E L P P L E I N S R V S
L K V G E T I E S G T V I F C D V L P G K E S L G Y
F V G V D M D N P I G N W D G R F D G V Q L C S
F A C V E S T I L L H I N D I I P A L S E S V T Q E R
R P P K L A F M S R G V G D K G S S S H N K P K A T G S T
S D P G N R N R S E L F Y T L N G S S V D S Q P Q S K
S K N T W Y I D E V A E D P A K S L T E I S T D
F D R S S P P L Q P P P V N S L T T E N R F H S L P F S
L T K M P N T N G S I G H S P L S L S A Q S V M E E L N
T A P V Q E S P P L A M P P G N S H G L E V G S
L A E V K E N P P F Y G V I R W I G Q P P G L N E V
L A G L E L E D E C A G C T D G T F R G T R Y F T
C A L K K A L F V K L K S C R P D S R F A S L Q P V S
N Q I E R C N S L A F G G Y L S E V V E E N T P P K
M E K E G L E I M I G K K K G I Q G H Y N S C Y L D
S T L F C L F A F S S V L D T V L L R P K E K N D V E Y Y
S E T Q E L L R T E I V N P L R I Y G Y V C A T K I M K L
R K I L E K V E A A S G F T S E E K D P E E F L N I L F
H H I L R V E P L L K I R S A G Q K V Q D C Y F Y Q I F
M E K N E K V G V P T I Q Q L L E W S F I N S N L K F A E
A P S C L I I Q M P R F G K D F K L F K K I F P S L E L
N I T D L L E D T P R Q C R I C G G L A M Y E C R E
C Y D D P D I S A G K I K Q F C K T C N T Q V H L H P
K R L N H K Y N P V S L P K D L P D W D W R H G C I P
C Q N M E L F A V L C I E T S H Y V A F V K Y G K D D
S A W L F F D S M A D R D G G Q N G F N I P Q V T P C P E
V G E Y L K M S L E D L H S L D S R R I Q G C A R R L L
C D A Y M C M Y Q S P T M S L Y K

Tag (**bold text**): N-terminal His
Protease cleavage site: TEV (ENLYFQ▼G)
CYLD (regular text): Start ***bold italics*** (amino acid residues 2-956)
Accession number: NP_056062

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Lot-specific COA version tracker: v1.0.0