

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of

<u>Enzyme description:-</u>	His-CYLD 2-956
<u>Clone number:-</u>	DU1834
<u>Source:-</u>	BL21 Recombinant
<u>Tag:-</u>	N-terminal His ₆ tag
<u>Purification method:-</u>	Ni ⁺⁺ -Sepharose
<u>Expression level:-</u>	10 mg/L

Calculated molecular mass:-

Monoisotopic	110485 Da
Average Mass	110553 Da
[cysteines reduced, methionines have not been oxidised]	

Theoretical pI:- 5.81

Purity:- 90%

Enzyme storage buffer:-

50 mM HEPES pH 7.5, 10% glycerol, 150mM NaCl, 1mM DTT

Storage temperature:- -80°C

Assay:-

Ub-Rho110-Gly cleavage assay monitored by Ex/Em 485/535 nm

Assay buffer:-

40 mM Tris pH 7.5, 100 mM NaCl, 5 mM DTT, 0.01% Triton X-100, 0.005% Ovalbumin, 0.5 μM Ub-Rho110-Gly

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Clone Data Sheet

His-CYLD

Protein His-CYLD residues 2-956
Synonyms
Clone Number Q9NQC7
Species Human
Accession Number Protein: Q9NQC7 DNA: NM015247.2
Tags N-terminal His₆ tag

Amino acid sequence of expressed protein MSYYHHHHHDYDIPTTENLYFQGAMGSSSGLWSQEKVTSPLYWEERIFYL
LLQECSVTDKQTQKLLKVPKGSIGQYIQDRSVGHSRIPSAKGKKNQIGLK
ILEQPHAVLFDVDEKDVVEINEKFTELLAI TNCEERFSLFKNRNRLSKGL
QIDVGCVPVKVQLRSGEKFFPGVVRFRGPLLAERTVSGIFFGVLELEEGRG
QGFTDGVYQKQLFQCDEDCGVFVALDKLELIEDDDTALESYAGPGDTM
QVELPPELEINSRVSLKVGETIESGTVIFCDVLPGKESLGYFVGVDMNDPI
GNWDGRFDGVQLCSFACVESTILLHINDIIPALSESVTQERRPPKLAFMS
RGVGDKGSSSHNPKATGSTSDPGNRNRSELYTLNGSSVDSQPQSKSKN
TWYIDEVAEDPAKSLTEISTDFDRSSPPLQPPVNSLTENRFHSLPFSL
TKMPNTNGSIGHSPLSLSAQSVMEELNTAPVQESPLAMPNGNSHGLEVG
SLAEVKENPPFYGVIRWIGOPPGLNEVLAGLELEDECAGCTDGTFRGTRY
FTCALKKALFVKLKSCRPDSPFASLQPVSNQIERCNLSLAFGGYLSEVVEE
NTPPKMEKEGLEIMIGKKKGIQGHYNLCYLDSTLFCFAFSSVLDTVLLR
PKEKNDVEYYSETQELLRTEIVNPLRIYGYVCATKIMKLRKILEKVEAAS
GFTSEEKDPPEFLNLFHHILRVEPLLKIRSAGQKVQDCYFYQIFMEKNE
KVGVPYTIQQLLEWSFINSNLKFAEAPSCLIIQMPRFGKDFKLFKKIFPSL
ELNITDLLEDTPRQCRI CGGLAMYECRECYDDPDISAGKIQFCCKTCNTQ
VHLHPKRLNHKYNPVSLPKDLPDWDWRHGCIPCQNMELFAVLCIETSHYV
AFVKYKDDSAWLFFDSMADRDGGQNGFNIPQVTPCPEVGEYKMSLEDL
HSLDSRRIQGCARRLLCDAYMCMYQSPTMSLYK

Native sequence CYLD 2-956 in bold
Protease cleavage TEV site underlined
Cloning sites BamH1

DNA sequence of insert

GGATCCAGTTCAGGCTTATGGAGCCAAGAAAAAGTCACTTCACCCTACTG
GGAAGAGCGGATTTTTTACTTGCTTCTTCAAGAATGCAGCGTTACAGACA
AACAAACACAAAAGCTCCTTAAAGTACCGAAGGGAAGTATAGGACAGTAT
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GAAGATGTCTCTGGAAGACCTGCATTCTTGGACTCCAGGAGAATCCAAG

GCTGTGCACGAAGACTGCTTTGTGATGCATATATGTGCATGTACCAGAGT
CCAACAATGAGTTTGTACAAATAAGGATCC