

UCHL3 [GST-tagged]

Deconjugating enzyme: Deubiquitylase

Alternate Name: Ubiquitin carboxyl terminal hydrolase isozyme L3

Cat. No. 64-0027-050
Lot. No. 30070

Quantity: 50 µg
Storage: -70°C



FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 2

Background

Deconjugating enzymes (DCEs) are proteases that process ubiquitin or ubiquitin-like gene products, reverse the modification of proteins by a single ubiquitin or ubiquitin-like protein (UBL) and remodel polyubiquitin (or poly-UBL) chains on target proteins (Reyes-Turcu *et al.*, 2009). The deubiquitylating – or deubiquitinating – enzymes (DUBs) represent the largest family of DCEs and regulate ubiquitin dependent signalling pathways. The activities of the DUBs 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 proteins (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. Ubiquitin carboxyl-terminal hydrolase L3 (UCHL3) is a member of the cysteine protease enzyme family and cloning of the human gene was first described by Wilkinson *et al.* (1989). The UCH sub-family of DUBs consists of four members: UCHL1, UCHL3, UCHL5 and BRCA1-associated protein-1 (BAP1) with UCHL3 sharing 54% homology with UCHL1 (Day and Thompson, 2010). Unlike other UCHs, UCHL3 can function as a C-terminal hydrolase for both NEDD8 and ubiquitin. UCHL3 may play a physiologically significant role in the

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Physical Characteristics

Species: human

Source: *E. coli*

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: ~53 kDa

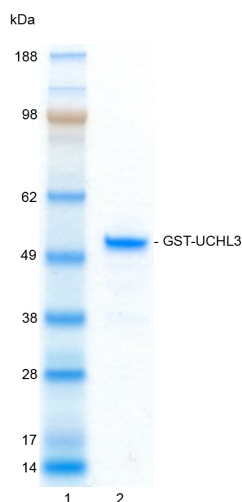
Purity: >95% by InstantBlue™ SDS-PAGE

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 GST-UCHL3



Protein Identification:
Confirmed by mass spectrometry.

Deubiquitylase Enzyme Assay:
The activity of GST-UCHL3 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 GST-UCHL3 was compared confirming the deubiquitylating activity of GST-UCHL3.



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Lot-specific COA version tracker: v1.0.0

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Background

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cleavage of the C-terminus of NEDD8, which is required for NEDD8 to conjugate to target proteins (Wada *et al.*, 1998). There is accumulating evidence suggesting that the expression and activity of UCH enzymes in cancers are abnormal. Increased levels of both UCHL1 and UCHL3 mRNA are associated with early tumour recurrence of invasive breast cancer and poor prognosis (Fang *et al.*, 2010).

References:

Day IN, Thompson RJ (2010) UCHL1 (PGP 9.5): neuronal bio-marker and ubiquitin system protein. *Prog Neurobiol* **90**, 327-362.

Fang Y, Fu D, Shen XZ (2010) The potential role of ubiquitin c-terminal hydrolases in oncogenesis. *Biochim Biophys Acta* **1806**, 1-6.

Komander D, Clague MJ, Urbe S (2009) Breaking the chains: structure and function of the deubiquitinases. *Nat Rev Mol Cell Biol* **10**, 550-563.

Reyes-Turcu FE, Ventii KH, Wilkinson KD (2009) Regulation and cellular roles of ubiquitin-specific deubiquitinating enzymes. *Ann Rev Biochem* **78**, 363-397.

Wada H, Kito K, Caskey LS, Yeh ET, Kamitani T (1998) Cleavage of the C-terminus of NEDD8 by UCH-L3. *Biochem Biophys Res Commun* **251**, 688-692.

Wilkinson KD, Lee KM, Deshpande S, Duerksen-Hughes P, Boss JM, Pohl J (1989) The neuron-specific protein PGP 9.5 is a ubiquitin carboxyl-terminal hydrolase. *Science* **246**, 670-673.

Physical Characteristics

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Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYY
IDGDVKLTQSMAIRYIADKHNMLGGCP
KERAEISMLEGAVLDIRYGVSR IAYSKD
FETLKVDFLSKLPEMLKMFEDRLCHKTYLNGD
HVTHPDFMLYDALDVVLYMDPMCLDAFP
KLVCFKKRIEAIPOIDKYLKSSKYIAW
PLQGWAQTFGGGDHPPKSDLEVLFGGPLG
SPGIPGSTRAAAMEGQRWLPLEANPEVTNQ
FLKQLGLHPNWQFVDVYGMDELLSMVPRPV
CAVLLLLFPITEKYEVEFRTEEEEEKIKSQGD
VTSSVYFMKQTISNACGTIGLIHAIANNKDK
MHFESGSTLKKFLEESVSMSP EERARYLENY
DAIRVTHETSAHEGQTEAPSIDEKVDLHFI
ALVHVDGHLYLELDGRKPPFINHGETSDETLLE
DAIEVCKKFMERDPDELRFNAIALSAA

Tag (**bold text**): N-terminal GST

Protease cleavage site: PreScission™ (**LEVLFG▼GP**)

UCHL3 (regular text): Start **bold italics** (amino acid residues 1-230)

Accession number: AAH18125



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