UCHL5 [GST-tagged]

Deconjugating enzyme: Deubiquitylase

Alternate Names: AD-019, CGI-70, UBL5, UCH37

Cat. No.	**64-0008-050**	Quantity:	50 µg
Lot. No.	**30069**	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 signaling 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 L5 (UCHL5) is a member of the cysteine protease enzyme family and cloning of the human gene was first described by Wicks *et al.* (2005). The deubiquitylating activity of the proteasome has been attributed to the action of three deubiquitylases: UCHL5, USP14 and RPN11, which are all localised in the 19S regulatory particle. It has been reported that loss of both UCHL5 and USP14 leads to the accumulation of polyubiquitylated proteins and an inhibition of protein degradation without altering the structure or catalytic properties of the proteasome (D'Arcy *et al.*, 2011). UCHL5 has been reported to interact with

Continued on page 2

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: ~64 kDa

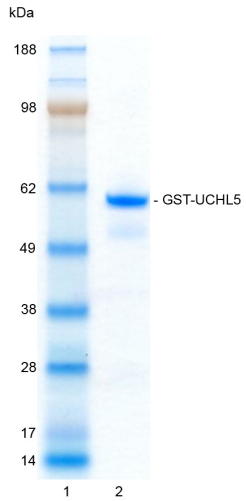
Purity: >88% by InstantBlue™ SDS-PAGE

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-UCHL5



Protein Identification:
Confirmed by mass spectrometry.

Deubiquitylase Enzyme Assay:
The activity of GST-UCHL5 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-UCHL5 was compared confirming the deubiquitylating activity of GST-UCHL5.

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Lot-specific COA version tracker: v1.0.0

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Background

Continued from page 1

the inhibitory protein SMAD7 by regulating transforming growth factor-β (TGF-β) receptor ubiquitylation, stability and transcription (Sacco *et al.*, 2010). The disruption of components in the TGF-β signalling cascade is a common occurrence in human cancers (Fang *et al.*, 2010). The activity of UCHL5 (and UCHL3) has been shown to be upregulated in the majority of tumour tissues compared to the adjacent normal tissues pointing to a specific role of these enzymes in the regulation of cell function and proliferation in different conditions, lending further support to the idea that UCHL5 (plus other deubiquitylases such as USP7 and USP9X) may constitute an interesting new target for the development of anticancer drugs (Rolen *et al.*, 2006)

References:

D'Arcy P, Brnjic S, Olofsson MH, Fryknas M, Lindsten K, De Cesare M, Perego P, Sadeghi B, Hassan M, Larsson R, Linder S (2011) Inhibition of proteasome deubiquitinating activity as a new cancer therapy. *Nat Med* **17**, 1636-1640.

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.

Rolen U, Kobzeva V, Gasparjan N, Ovaa H, Winberg G, Kisseljov F, Masucci MG (2006) Activity profiling of deubiquitinating enzymes in cervical carcinoma biopsies and cell lines. *Mol Carcinog* **45**, 260-269.

Sacco JJ, Coulson JM, Clague MJ, Urbe S (2010) Emerging roles of deubiquitinases in cancer-associated pathways. *IUBMB Life* **62**, 140-157.

Wicks SJ, Haros K, Maillard M, Song L, Cohen RE, Dijke PT, Chantry A (2005) The deubiquitinating enzyme UCH37 interacts with Smads and regulates TGF-beta signalling. *Oncogene* **24**, 8080-8084.

Physical Characteristics

Continued from page 1

Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYY
IDGDVKLTQSMAIIRYIADKHNMLGGCPKER
AEISMLEGAVLDIRYGVSRIAYSKDFETLKVD
FLSKLPEMLKMFEDRLCHKTYLNGDHVTHP
DFMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPQIDKYLKSSKYIAWPLQGWQATF
GGGDHPPKSDLEVLFQGPLGS***M***TGNAGEW
CLMESDPGVFTELIKGFGCRGAQVEEI
WSLEPENFEKLKPVHGLIFLFKWQPGEEP
AGSVVQDSRLDTIFFAKQVINNACATQAIVS
VLLNCTHQDVHLGETLSEFKEFSQSFDAAMK
GLALSNSDVIRQVHNSFARQQMFEFDTKTSA
KEEDAFHFVSYVPVNGRLYELDGLREGPIDL
GACNQDDWISAVRPVIEKRIQKYSEGEIRF
NLMAIVSDRKMIYEQKIAELQRQ
LAEEPMDTDQGNSMLSAIQSEVAKNQM
LIEEEVQKLKRYKIENIRRKHNYLPFIMELLK
TLAEHQQLIPLVEKAKEKQNAKKAQETK

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
UCHL5 (regular text): Start ***bold italics*** (amino acid residues 1-328)
Accession number: AAH15521.1

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