USP5 [6His-tagged]

Deubiquitylating Enzyme

Alternate Names: Isopeptidase T, ISOT

Cat. No. **64-0002-050**
Lot. No. **1735**

Quantity: 50 µg
Storage: -70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 2

Background

The Deubiquitylating enzymes (DUBs) 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 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. Ubiquitin specific processing protease 5 (USP5) is a member of the cysteine protease enzyme family and cloning of the human gene was first described by Wilkinson *et al*. (1995). USP5 protein contains an N-terminal zinc finger ubiquitin-binding domain (ZNF-UBP), a ubiquitin-specific processing protease (UBP) domain containing the active-site cys and his boxes, and two ubiquitin-associated domains (UBA1 and UBA2) (Reyes-Turcu *et al*., 2006). Crystal structures of the ZNF-UBP domain have revealed a deep binding pocket where the C-terminal diglycine motif of ubiquitin is inserted explaining the specificity of USP for an unmodified C-terminus on the proximal subunit of polyubiquitin (Reyes-Turcu *et al*., 2006). Recently USP5 has been identified in association with the 26S proteasome alongside other proteins

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Physical Characteristics

Species: human

Source: *E. coli* 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: ~98 kDa

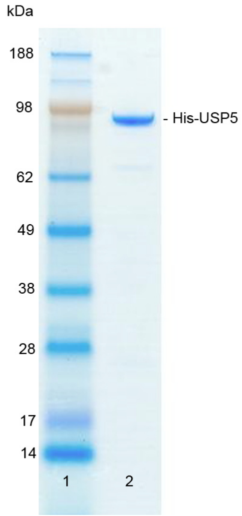
Purity: >90% by InstantBlue™ SDS-PAGE

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-USP5



Protein Identification:
Confirmed by mass spectrometry.

Deubiquitylating Enzyme Assay:
The activity of His-USP5 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-USP5 was compared confirming the deubiquitylating activity of His-USP5.

Lot-specific COA version tracker: v1.0.0

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CERTIFICATE OF ANALYSIS Page 2 of 2

Background

Continued from page 1

known in complex as the UBL interactome (Besche *et al*., 2009).

References:

Besche H, Haas W, Gygi S, Goldberg A (2009) Isolation of mammalian 26S proteasomes and p97/VCP complexes using the ubiquitin-like domain from HHR23B reveals novel proteasome-associated proteins. *Biochemistry*.**48**, 2538-49.

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

Reyes-Turcu FE, Horton JR, Mullally JE, Heroux A, Cheng X, Wilkinson KD (2006) The ubiquitin binding domain ZnF UBP recognizes the C-terminal diglycine motif of unanchored ubiquitin. *Cell* **124**, 1197-208.

Wilkinson KD (1995) Roles of ubiquitinylation in proteolysis and cellular regulation. *Annu Rev Nutr* **15**, 161-89.

Physical Characteristics

Continued from page 1

Protein Sequence:

MGSSHHHHHHSSGLEVLFQGPGSPNS
PLGS***M***AELSEEALLSVLPTIRVPK
AGDRVHKDECAFSFDTPESEGGLYIC
MNTFLGFGKQYVERHFNKTGQRVYLHL
RRTRRPKEEDPATGTGDPPRKKPTR
LAIGVEGGFDLSEEKFELDEDVKIVILP
DYLEIARDGLGGLPDIVRDRVTSAVEALL
SADSASRKQEVQAWDGEVRQVSKHAFS
LKQLDNPARIPPCGWKCSKCDMRENL
WLNLTDGSILCGRRYFDGSGGNNHAVE
HYRETGYPLAVKLGTITPDGADVYSYDED
DMVLDPSLAEHLSHFGIDMLKMQKTDK
TMTELEIDMNQRIGEWELIQESGVPLK
PLFGPGYTGIRNLGNSCYLNSVVQVLF
SIPDFQRKYVDKLEKIFQNAPTDPTQD
FSTQVAKLGHGLLSGEYSKPVPESGDG
ERVPEQKEVQDGIAPRMFKALIGKGHPEF
STNRQQDAQEFFLHLINMVERNCRSSENP
NEVFRFLVEEKIKCLATEKVKYTQRVDY
IMQLPVPMDAALNKEELLEYEEK
KRQAEEEKMALPELVRAQVPFSS
CLEAYGAPEQVDDFWSTALQAKS
VAVKTTRFASFPDYLVIQIKKFT
FGLDWVPKKLDVSIEMPEELDIS
QLRGTGLQPGEEELPDIAPPLVTPDEP
KGSLGFYGNEDEDSFCSPHFSSPTSP
MLDESVIIQLVEMGFPMDACRKAVYYT
GNSGAEAAMNWVMSHMDDPDFANPLIL
PGSSGPGSTSAAADPPPEDCVTTIVSMGF
SRDQALKALRATNNSLERAVDWIFSHID
DLDAEAAMDISEGRSAADSISESVPVGP
KVRDGPGKYQLFAFISHMGTSTMCGHYV
CHIKKEGRWVIYNDQKVCASEKPPKDLGY
IYFYQRVAS

Tag (**bold text**): N-terminal His
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
USP5 (regular text): Start ***bold italics*** (amino acid residues 1-858)
Accession number: P45974

Lot-specific COA version tracker: v1.0.0