USP4 [6His-tagged]

Deconjugating enzyme: Deubiquitylase

Alternate Names: Deubiquitinating enzyme 4, Ubiquitin carboxyl-terminal hydrolase 4, Ubiquitin-specific-processing protease 4, Ubiquitin thioesterase 4

Cat. No. 64-0001-050
Lot. No. 30031

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 4 (Ubiquitin Specific Protease 4; USP4) is a member of the cysteine protease enzyme family and cloning of the human gene was first described by Gupta *et al.* (1993). In 1995, USP4 was identified as a proto-oncogene related to USP6, showing a consistently elevated gene expression level in small cell tumours and lung adenocarcinomas suggesting that it may have a possible causative role in neoplasia (Gray *et al.*, 1995). USP4 has been implicated in a number of other processes, including protein quality control in the endoplasmatic

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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: ~111 kDa

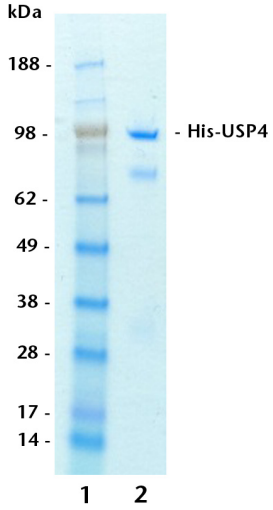
Purity: >76% by InstantBlue™ SDS-PAGE

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-USP4



Protein Identification:
Confirmed by mass spectrometry.

Deubiquitylating Enzyme Assay:
The activity of His-USP4 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-USP4 was compared confirming the deubiquitylating activity of His-USP4.

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Lot-specific COA version tracker: v1.0.0

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Background

Continued from page 1

reticulum and p53 and Wnt signalling. USP4 has also been reported to inhibit the kinase TAK1 that is ubiquitylated by the AKT regulator TRAF6 (Uras *et al.*, 2012).

References:

Gray DA, Inazawa J, Gupta K, Wong A, Ueda R and Takahashi T (1995) Elevated expression of Unph, a proto-oncogene at 3p21.3, in human lung tumors. *Oncogene* **10**, 2179-2183.

Gupta K, Copeland NG, Gilbert DJ, Jenkins NA and Gray DA (1993) Unp, a mouse gene related to the tre oncogene. *Oncogene* **8**, 2307-2310.

Komander D, Clague MJ and 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 and Wilkinson KD (2009) Regulation and cellular roles of ubiquitin-specific deubiquitinating enzymes. *Ann Rev Biochem* **78**, 363-397.

Uras IZ, List T and Nijman SM (2012) Ubiquitin-specific protease 4 inhibits mono-ubiquitination of the master growth factor signaling kinase PDK1. *PloS one* **7**, e31003.

Physical Characteristics

Continued from page 1

Protein Sequence:

MGSSHHHHHHSSGLEVLFQGPGS***M***AEGGGCR
ERPDAETQKSELGPLMRTTLQRGAQWYLID
SRWFKQWKKYVGFDSWDMYNVGEHNLFPG
PIDNSGLFSDPESQTLKEHLIDELDYVLVP
TEAWNKLLNWYGCVEGQQPIVRKVVEH
GLFVKHCKVEVYLLELKLCENSDPTNVLSCHF
SKADTIATIEKEMRKLFNIPAERETRL
WNKYMSNTYEQLSKLDNTVQDAGLYQGQV
LVIEPQNEDGTWPRQTLQSKSSTAPSRN
FTTSPKSSASPYSSVSASLIANGDSTSTCG
MHSSGVSRGGSGFSASYNCQEPPSSHIQPGL
CGLGNLGNTCFMNSALQCLSNTAPLTDYFLKDE
YEAEINRDNPLGMKGEIAEAYAELIKQMWSGR
DAHVAPRMFKTQVGRFAPQFSGYQQQDSQEL
LAFLLDGLHEDLNRVKKKPYLELKDANGRPDAV
VAKEAWENHRLRNDSVIVDTFHGLFKSTLVCPE
CAKVSVTFDPFCYLTLPLPLKKDRVMEVFLV
PADPHCRPTQYRVTVPLMGAVSDLCEALSRLS
GIAAENMVVADVYNHRFHKIFQMDEGLNHIMPRD
DIFVYEVCSTSVDGSECVTLPVYFRERK
SRPSSTSSASALYGQPLLLSVPKHKLTLESLY
QAVCDRISRYVKQPLPDEFGSSPLEPGACNG
SRNSCEGEDEEEMEHQEEGKEQLSETEGS
GEDEPGNDPSETTQKKIKGQPCPKRLFTFSLVN
SYGTADINSLAADGKLLKLNSRSTLAMDWDSE
TRRLYYDEQESEAYEKHVSMLQPQKKKKTTVAL
RDCIELFTTMETLGEHDPWYCPNCKKHQQATK
KFDLWSLPKILVVHLKRFSYNRYWRDKLDTV
VEFPIRGLNMSEFVCNLSARPYVYDLIAVSNHYG
AMGVGHYTAYAKNKLNGKWYYFDDSNVSLASEDQ
IVTKAAYVLFYQRRDDEFYKTPSLSSSGSSDGG
TRPSSSQQGFGDDEACSMDTN

Tag (**bold text**): N-terminal His
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
USP4 (regular text): Start ***bold italics*** (amino acid residues 1-963)
Accession number: NP_003354

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Lot-specific COA version tracker: v1.0.0