USP2 (isoform 4) [GST-tagged]

Deconjugating enzyme: Deubiquitylase

Alternate Names: 41 kDa ubiquitin specific protease, UBP41

Cat. No. **64-0014-050**
Lot. No. **30013**

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 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 specific protease 2 (USP2) is a member of the cysteine protease enzyme family and cloning of the human gene was first described by Baek *et al.* (1997). USP2 influences the nuclear translocation of NF-κB transcription factors in response to inflammation, injury and other environmental changes. In resting cells, NF-κB is bound to the inhibitory protein IκBα, and therefore maintained in an inactive state in the cytoplasm. The proteasomal degradation of IκBα is prevented by USP2 deubiquitylase activity. This regulates the expression of genes that are involved in immune responses, cell proliferation,

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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: ~73 kDa

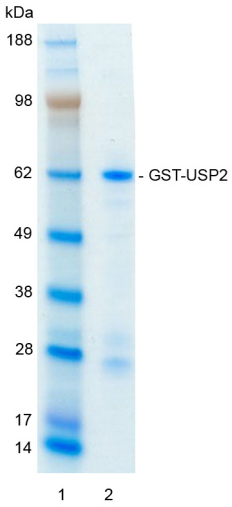
Purity: >68% by InstantBlue™ SDS-PAGE

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-USP2



Protein Identification:
Confirmed by mass spectrometry.

Deubiquitylase Enzyme Assay:
The activity of GST-USP2 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-USP2 was compared confirming the deubiquitylating activity of GST-USP2.

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Lot-specific COA version tracker: v1.0.0

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Background

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differentiation and apoptosis (Metzig *et al.*, 2011). Deregulation of NF-κB activity is involved in the pathology of many diseases including chronic inflammation and cancer. USP2 expression itself is frequently downregulated in breast carcinomas (Metzig *et al.*, 2011). USP2 deubiquitylase activity induces cell death via the apoptosis inducing factor (AIF). AIF is a mitochondrial oxidoreductase that becomes truncated when the cell is under stress and the truncated AIF (tAIF) becomes translocated into the nucleus, and induces caspase-independent cell death. USP2 deubiquitylates and stabilizes tAIF, thus promoting AIF-mediated cell death. In contrast, the E3 ligase CHIP ubiquitylates and destabilizes tAIF, thus preventing cell death (Oh *et al.*, 2011).

References:

Baek SH, Choi KS, Yoo YJ, Cho JM, Baker RT, Tanaka K, Chung CH (1997) Molecular cloning of a novel ubiquitin-specific protease, UBP41, with isopeptidase activity in chick skeletal muscle. *J Biol Chem* **272**, 25560-25565.

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

Metzig M, Nickles D, Falschlehner C, Lehmann-Koch J, Straub BK, Roth W, Boutros M (2011) An RNAi screen identifies USP2 as a factor required for TNF-alpha-induced NF-kappaB signaling. *Int J Cancer* **129**, 607-618.

Oh KH, Yang SW, Park JM, Seol JH, Iemura S, Natsume T, Murata S, Tanaka K, Jeon YJ, Chung CH (2011) Control of AIF-mediated cell death by antagonistic functions of CHIP ubiquitin E3 ligase and USP2 deubiquitinating enzyme. *Cell Death Differ* **18**, 1326-1336.

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

Physical Characteristics

Continued from page 1

Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYYIDGD
VKLTQSMAIIRYIADKHNMLGGCPKERAEISM
LEGAVLDIRYGVSRIAYSKDFETLKVDFL
SKLPEMLKMFEDRLCHKTYLNGDHVTHPD
FMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPQIDKYLKSSKYIAWPLQGWQATFG
GGDHPPKSDLEVLFQGPLGSPNSRVD***M***RTSYT**
VTLPEDPPAAPFPALAKELRPRSPLSPSLLL
STFVGLLLNKAKNSKSAQGLAGLRNLGNTCF
MNSILQCLSNTRELRDYCLQRLYMRDLHHGS
NAHTALVEEFAKLIQTIWTSSPNDVVSPSEFK
TQIQRYAPRFVGYNQQDAQEFLRFLLDGLH
NEVNRVTLRPKSNPENLDHLPDDEKGRQM
WRKYLEREDSRIGDLFVGQLKSSLTCTDC
GYCSTVFDPFWDLSLPIAKRGYPEVTLMDC
MRLFTKEDVLDGDEKPTCCRCRGRKRCIK
KFSIQRFPKILVLHLKRFSESRIRTSKLT
TFVNFPLRDLDLREFASENTNHAVYNLYAVS
NHSGTTMGGHYTAYCRSPGTGEWHTFNDSS
VTPMSSSQVRTSDAYLLFYELASPPSRM

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
USP2 (regular text): Start ***bold italics*** (amino acid residues 1-396)
Accession number: NP_741994
UniProt number: O75604-4

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