USP9x CD(1554-1995) [GST-tagged]

Deconjugating enzyme: Deubiquitylase

Alternate Names: Deubiquitinating enzyme FAF-X, DFFRX, EC 3.1.2.15, Fat facets protein related, X-linked, Ubiquitin thiolesterase FAF-X, Ubiquitin-specific processing protease FAF-X, Ubiquitin-specific protease 9, X chromosome

Cat. No. **64-0017-050** Quantity: 50 µg
Lot. No. **30060** Storage: -70˚C

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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 9, X chromosome (USP9X) is a member of the cysteine protease enzyme family and cloning of the human gene was first described by Jones *et al.* (1996). USP9X is a deubiquitylase involved both in the processing of many different ubiquitin precursors and ubiquitylated proteins. USP9X is known to stabilise β-catenin, thereby enhancing pro-survival Notch and WNT signalling, as well as the self-renewal of embryonic stem cell-derived neural progenitors. USP9X also enhances transforming growth factor-β (TGF-β) signalling via deubiquitylation of TGF-β receptors and SMAD intracellular medi-

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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: ~79 kDa

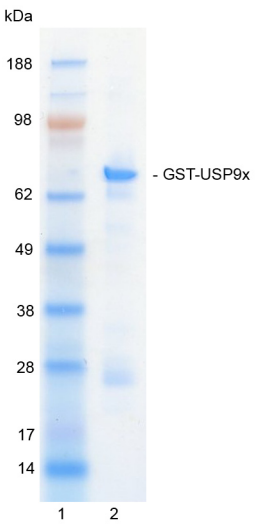
Purity: >78% by InstantBlue™ SDS-PAGE

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-USP9x



Protein Identification:
Confirmed by mass spectrometry.

Deubiquitylase Enzyme Assay:
The activity of GST-USP9x 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-USP9x was compared confirming the deubiquitylating activity of GST-USP9x.

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Lot-specific COA version tracker: v1.0.0

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Background

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ators (Vucic *et al.*, 2011). USP9X acts by removing monoubiquitin from SMAD4, thereby permitting its association with phospho-SMAD2 and subsequent activation of TGF-β/SMAD-responsive gene targets. USP9X has also been shown to control AMP-activated protein kinase (AMPK)-related kinase activity through direct removal of non-canonical K29 and/or K33-linked ubiquitin chains (Sacco *et al.*, 2010). USP9X can also stabilise levels of myeloid cell leukemia sequence 1 protein (MCL1) and thereby promotes cell survival. USP9X binds MCL1 and removes the Lys 48-linked polyubiquitin chains that normally mark MCL1 for proteasomal degradation. Increased USP9X expression correlates with increased MCL1 protein in human follicular lymphomas and diffuse large B-cell lymphomas. Moreover, patients with multiple myeloma overexpressing USP9X have a poor prognosis. These results identify USP9X as a prognostic and therapeutic target, and they show that deubiquitylases may stabilise labile oncoproteins in human malignancies (Schwickart *et al.*, 2010). USP9X also interacts and deubiquitylates α-synuclein *in vitro* and *in vivo*. α-Synuclein is central to the pathogenesis of Parkinson disease (PD). Drugs that modulate USP9X activity, together with enhancers of autophagy or proteasomal activity, may help decrease the levels of α-synuclein and provide a novel therapeutic strategy to treat α-synucleinopathies (Rott *et al.*, 2011)

References:

Jones MH, Furlong RA, Burkin H, Chalmers IJ, Brown GM, Khwaja O, Affara NA (1996) The *Drosophila* developmental gene fat facets has a human homologue in Xp11.4 which escapes X-inactivation and has related sequences on Yq11.2. *Hum Mol Genet* **5**, 1695-1701.

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.

Rott R, Szargel R, Haskin J, Bandopadhyay R, Lees AJ, Shani V, Engelender S (2011) alpha-Synuclein fate is determined by USP9X-regulated monoubiquitination. *Proc Nat Acad Sci USA* **108**, 18666-18671.

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

Schwickart M, Huang X, Lill JR, Liu J, Ferrando R, French DM, Maecker H, O'Rourke K, Bazan F, Eastham-Anderson J, Yue P, Dornan D, Huang DC, Dixit VM (2010) Deubiquitinase USP9X stabilizes MCL1 and promotes tumour cell survival. *Nature* **463**, 103-107.

Vucic D, Dixit VM, Wertz IE (2011) Ubiquitylation in apoptosis: a post-translational modification at the edge of life and death. *Nat Rev Mol Cell Biol* **12**, 439-452.

Physical Characteristics

Continued from page 1

Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYYIDGD
VKLTQSMAIIRYIADKHNMLGGCPKERAEISM
LEGAVLDIRYGVSRIAYSKDFETLKVDFL
SKLPEMLKMFEDRLCHKTYLNGDHVTHPD
FMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPQIDKYLKSSKYIAWPLQGWQATFG
GGDHPPKSDLEVLFQGPLGSLEVLFQGP***K***G
FVGLKNAGATCYMNSVIQQLYMIPSIRNGI
LAIEGTGSDVDDDMSGDEKQDNESNVDPRD
DVFGYPQQFEDKPALSKTEDRKEYNIGVL
RHLQVIFGHLAASRLQYYVPRGFWKQFRL
WGEPVNLREQHDALEFFNSLVDSLDEALKA
LGHPAMLSKVLGGSFADQKICQGCPHRYE
CEESFTTLNVDIRNHQNLLDSLEQYVKGDLLE
GANAYHCEKCNKKVDTVKRLLIKKLPPVLAI
QLKRFDYDWERECAIKFNDYFEFPRELDMEPY
TVAGVAKLEGDNVNPESQLIQQSEQSESETAG
STKYRLVGVLVHSGQASGGHYYSYIIQRNG
GDGERNRWYKFDDGDVTECKMDDDEEMKNQCF
GGEYMGEVFDHMMKRMSYRRQKRWWNAYILFY
ERMDTIDQDDELIRYISELAITTRPHQIIMP
SAIERSVRKQN

Tag (**bold text**): N-terminal GST
Protease cleavage site: 2x PreScission™ (LEVLFQ▼GP)
USP9x (regular text): Start ***bold italics*** (amino acid residues 1554-1995)
Accession number: NP_001034680

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