USP20 [GST-tagged]

Deconjugating enzyme: Deubiquitylase

Alternate Names: KIAA1003hVDU2, LSFR3A, pVHL-interacting deubiquitinating enzyme 2, ubiquitin carboxyl-terminal hydrolase 20, ubiquitin specific protease 20, ubiquitin thioesterase 20, VDU2

Cat. No. 64-0039-050 Quantity: 50 µg Lot. No. 30134 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 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 specific protease 20 (USP20) is a member of the cysteine protease enzyme family and cloning of the gene was first described by Nagase et al. (1999). USP20 has been shown to specifically deubiquitylate and stabilise Hypoxia-inducible factor (HIF)-1α, increasing expression of HIF-1α targeted genes, such as vascular endothelial growth factor (VEGF) (Li et al., 2005). USP20 also deubiquitylates tumour necrosis receptor-associated factor 6 (TRAF6) and Tax and suppresses interleukin 1β (IL-1β) and Tax-induced

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Physical Characteristics

Protein Sequence: Please see page 2 Species: human

Source: Insect Sf21

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: ~128.7 kDa

Purity: >70% by InstantBlue™ SDS-PAGE

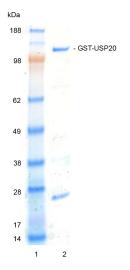
Stability/Storage: 12 months at -70°C;

aliquot as required

Quality Assurance

Purity:

4-12% gradient SDS-PAGE InstantBlue™ staining Lane 1: MW markers Lane 2: 1 µg GST-USP20



Protein Identification:

Confirmed by mass spectrometry.

Deubiquitylase Enzyme Assay:

The activity of GST-USP20 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-USP20 was compared confirming the deubiquitylating activity of GST-USP20.



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Lot-specific COA version tracker: v1.0.0

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CERTIFICATE OF ANALYSIS Page 2 of 2

Background

Continued from page 1

NF-kB activation. Recent evidence has shown that USP20 overexpression may impede the proliferation of human T cell leukaemia virus type 1/adult T cell leukaemia (HTLV-1/ATL) cells and suggest that screening for small-molecule compounds to enhance USP20 deubiquitylase activity may unveil new agents that are useful for treating adult T cell leukaemia (Yasunaga et al., 2011)

References:

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.

Li Z, Wang D, Messing EM and Wu G (2005) VHL protein-interacting deubiquitinating enzyme 2 deubiquitinates and stabilizes HIF-1alpha. *EMBO Rep* **6**, 373-378.

Nagase T, Ishikawa K, Suyama M, Kikuno R, Hirosawa M, Miyajima N, et al. (1999) Prediction of the coding sequences of unidentified human genes. XIII. The complete sequences of 100 new cDNA clones from brain which code for large proteins in vitro. DNA research: an international journal for rapid publication of reports on genes and genomes 6, 63-70.

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

Yasunaga J, Lin FC, Lu X and Jeang KT (2011) Ubiquitin-specific peptidase 20 targets TRAF6 and human T cell leukemia virus type 1 tax to negatively regulate NF-kappaB signaling. *J Virol* 85, 6212-6219

Physical Characteristics

Continued from page 1

Protein Sequence:

MSPILGYWKIKGLVOPTRLLLEYLEEKYEEH LYERDEGDKWRNKKFELGLEFPNLPYY **IDGDVKLTQSMAIIRYIADKHNMLGGCP** KERAEISMLEGAVLDIRYGVSRIAYSKD **FETLKUDFLSKLPEMLKMFEDRLCHK** TYLNGDHVTHPDFMLYDALDVVLYMDPM CLDAFPKLVCFKKRIEAIPQIDKYLKSSKY IAWPLQGWQATFGGGDHPPKSDLEVLFQG PLGSMGDSRDLCPHLDSIGEVTKEDLLLK SKGTCQSCGVTGPNLWACLQVACPYVGCGES FADHSTIHAQAKKHNLTVNLTTFRLWCY ACEKEVFLEQRLAAPLLGSSSKFSEQDSPPP SHPLKAVPIAVADEGESESEDDDLKPRGLT GMKNLGNSCYMNAALOALSNCPPLTOF FLECGGLVRTDKKPALCKSYQKLVSEVWHK KRPSYVVPTSLSHGIKLVNPMFRGYAQQDTQE FLRCLMDQLHEELKEPVVATVALTEARDSDSS DTDEKREGDRSPSEDEFLSCDSSS DRGEGDGQGRGGGSSQAETELLIPDEA GRAISEKERMKDRKFSWGQQRTNSEQVDE DADVDTAMAALDQPAEAQPPSPRSSSP CRTPEPDNDAHLRSSSRPCSPVHH HEGHAKLSSSPPRASPVRMAPSYVLKKAQV LSAGSRRRKEQRYRSVISDIFDGSILSLVQ CLTCDRVSTTVETFODLSLPIPGKEDLAKLH SAIYQNVPAKPGACGDSYAAQGWLAFIVEYIR RFVVSCTPSWFWGPVVTLEDCLAAFFAADELK GDNMYSCERCKKLRNGVKYCKVLRLPEIL CIHLKRERHEVMYSEKINSHVSEPLEGLDL RPFLAKECTSQITTYDLLSVICHHGTAGS GHYIAYCQNVINGQWYEFDDQYVTEVHETV VQNAEGYVLFYRKSSEEAMRERQQVVSLAAM REPSLLRFYVSREWLNKFNTFAEPGPITNQT FLCSHGGIPPHKYHYIDDLVVILPONVWEH LYNRFGGGPAVNHLYVCSICQVEIEALAKRR RIEIDTFIKLNKAFQAEESPGVI YCISMQWFREWEAFVKGKDNEPPG PIDNSRIAQVKGSGHVQLKQGADYGQI SEETWTYLNSLYGGGPEIAIRQSVAQPLGPEN LHGEQKIEAETRAV

Tag (bold text): N-terminal GST

Protease cleavage site: PreScission™ (<u>LEVLFQ▼GP</u>) USP20 (regular text): Start **bold italics** (amino acid

residues 1-913)

Accession number: AAH39593



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