USP36 CD(81-461) [GST-tagged]

Deconjugating enzyme: Deubiquitylase

Alternate Names: FLJ12851, KIAA1453

 Cat. No.
 64-0006-050
 Quantity:

 Lot. No.
 30065
 Storage:

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CERTIFICATE OF ANALYSIS Page 1 of 2

Protein Sequence: Please see page 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 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 modifications through chain editing to rescue proteins from proteasomal degradation or to influence cell signaling events (Komander et al., 2009). There are two main classes of DUB; cysteine proteases and metalloproteases. Ubiquitin specific protease 36 (USP36) is a member of the cysteine protease enzyme family and cloning of the human gene was first described by Nagase et al. (2000). USP36 is primarily localized to the nucleoli, is required to maintain normal nucleolar structure and is highly expressed in skeletal muscle and testis. Nucleophosmin and fibrillarin are two nucleolar proteins that have been shown to undergo ubiquitylation, and are substrates for USP36. The deubiquitylating activity of USP36 controls transcriptional regulation and ribosome biogenesis in response to the changes in environmental conditions (Endo et al., 2009). USP36 is also known to deubiqui-

Physical Characteristics

50 µg

-70°C

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: ~69 kDa

Purity: >84% by InstantBlue™ SDS-PAGE

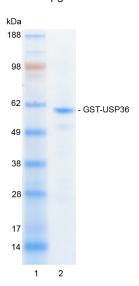
Stability/Storage: 12 months at -70°C;

aliquot as required

Quality Assurance

Purity:

4-12% gradient SDS-PAGE InstantBlue™ staining lane 1: MW markers lane 2: 1 μg GST-USP36



Protein Identification:

Confirmed by mass spectrometry.

Deubiquitylase Enzyme Assay:

The activity of GST-USP36 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-USP36 was compared confirming the deubiquitylating activity of GST-USP36.

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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

tylate histone H2B and functions in gene silencing which is a common mechanism within stem cells in order to repress the premature expression of key differentiation genes, including Notch target genes (Buszczak *et al.*, 2009). USP36 is over-expressed in ovarian cancer cells, and may act as an oncogene by suppressing differentiation (Li *et al.*, 2008)

References:

Buszczak M, Paterno S, Spradling AC (2009) *Drosophila* stem cells share a common requirement for the histone H2B ubiquitin protease scrawny. *Science* **323**, 248-251.

Endo A, Matsumoto M, Inada T, Yamamoto A, Nakayama KI, Kitamura N, Komada M (2009) Nucleolar structure and function are regulated by the deubiquitylating enzyme USP36. *J Cell Sci* **122**, 678-686.

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

Li J, Olson LM, Zhang Z, Li L, Bidder M, Nguyen L, Pfeifer J, Rader JS (2008) Differential display identifies overexpression of the USP36 gene, encoding a deubiquitinating enzyme, in ovarian cancer. *Int J Med Sci* 5, 133-142.

Nagase T, Kikuno R, Ishikawa K, Hirosawa M, Ohara O (2000) Prediction of the coding sequences of unidentified human genes. XVII. The complete sequences of 100 new cDNA clones from brain which code for large proteins *in vitro*. *DNA Res* 7, 143-150.

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:

MSPILGYWKIKGLVOPTRLLLEYLEEKYEEH LYERDEGDKWRNKKFELGLEFPNLPYY **IDGDVKLTQSMAIIRYIADKHNMLGGCP** KERAEISMLEGAVLDIRYGVSRIAYSKD **FETLKVDFLSKLPEMLKMFEDRLCHKTYLNGD** HVTHPDFMLYDALDVVLYMDPMCLDAFP KLVCFKKRIEAIPQIDKYLKSSKYIAWPLQG WQATFGGGDHPPKSDLEVLFQGPLGSARRQG SEHTYESCGDGVPAPQKVLFPTERLSLRW ERVFRVGAGLHNLGNTCFLNATIQCLTYT PPLANYLLSKEHARSCHOGSFCMLCVMON HIVOAFANSGNAIKPVSFIRDLKKIARHFRF GNQEDAHEFLRYTIDAMQKACLNGCAKL DRQTQATTLVHQIFGGYLRSRVKCSVCKSVS DTYDPYLDVALEIRQAANIVRALELFVKADV LSGENAYMCAKCKKKVPASKRFTIHRTSNV LTLSLKRFANFSGGKITKDVGYPEFLNIRPYM SQNNGDPVMYGLYAVLVHSGYSCHAGHYY CYVKASNGQWYQMNDSLVHSSNVKVVLNQQAY VLFYLRIPGSKKSPEGLISRTGSSSLPGRPS VIPDHSKKNIGNGI

Tag (**bold text**): N-terminal GST

Protease cleavage site: PreScission™ (<u>LEVLFQ▼GP</u>) USP36 (regular text): Start **bold italics** (amino acid

residues 81-461)

Accession number: AAH27992.1



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