USP8 [untagged]

Deconjugating enzyme

Alternate Names: Ubiquitin carboxyl-terminal hydrolase 8, Ubiquitin thioesterase 8, Ubiquitin-specific-processing protease 8, Ubiquitin isopeptidase Y

Cat. No. **64-0053-050**
Lot. No. **30212**

Quantity: 50 µg
Storage: -70˚C

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NOT FOR USE IN HUMANS

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 8 (USP8) is a member of the cysteine protease enzyme family and cloning of the gene was first described by Nomura *et al.* (1994). USP8 regulates the degradation of various transmembrane proteins at the sorting endosome by modulating the ubiquitin dynamics of both cargo and sorting proteins. USP8 interacts with signal transducing adaptor molecule (STAM) and stabilizes STAM and hepatocyte growth-factor-regulated substrate (Hrs), which together constitute the endosomal sorting complex required for transport (ESCRT) and govern the early steps of receptor trafficking en route to the lysosomes (De Ceuninck

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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: ~128 kDa

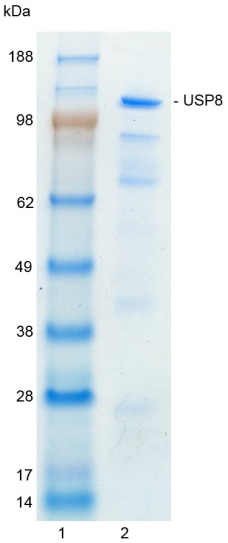
Purity: >66% by InstantBlue™ SDS-PAGE

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 USP8



Protein Identification:
Confirmed by mass spectrometry.

Deubiquitylase Enzyme Assay: The activity of USP8 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 USP8 was compared confirming the deubiquitylating activity of USP8.

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Lot-specific COA version tracker: v1.0.0

USP8 [untagged]

Deconjugating enzyme

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Background

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et al., 2013). The E3 ubiquitin ligase IDOL (inducible degrader of the LDLR) employs ESCRT complexes to recognise and traffic low-density lipoprotein receptor (LDLR) to lysosomes. IDOL is recruited to the plasma membrane by LDLR, promoting LDLR internalisation and facilitating LDLR degradation by shuttling it into the multivesicular body (MVB) protein-sorting pathway. USP8 acts downstream of IDOL to deubiquitylate LDLR and is required for LDLR entry into the MVB pathway (Scotti *et al.*, 2013; Sorrentino *et al.*, 2013). USP8 has also been shown to interact with and stabilise another E3 ubiquitin ligase called Ring Finger Protein 41 (RNF41) which is also known to be involved in the trafficking of various transmembrane proteins. USP8 is a known substrate of RNF41 whereby RNF41 redistributes and ubiquitylates USP8, thus reducing USP8 levels. Balanced reciprocal cross-regulation between RNF41 and USP8 decides if receptors are sorted for lysosomal degradation or recycling, this way regulating basal cytokine receptor levels (De Ceuninck *et al.*, 2013). Recent cell-based and *in vivo* work has shown that the inhibition of USP8 activity or reduction in USP8 expression can selectively kill non-small cell lung cancer (NSCLC) cells. USP8 suppression leads to the downregulation of multiple oncogenic receptor tyrosine kinase (RTK) receptors; EGFR, ERBB2, ERBB3, and MET. Based on this work, USP8 has been proposed as a potential therapeutic target for both gefitinib-resistant and -sensitive NSCLC cells (Byun *et al.*, 2013).

References:

Byun S, Lee SY, Lee J, Jeong CH, Farrand L, Lim S, *et al.* (2013) USP8 Is a Novel Target for Overcoming Gefitinib Resistance in Lung Cancer. *Clinical cancer research: an official journal of the American Association for Cancer Research* **19**, 3894-3904.

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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.

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

Scotti E, Calamai M, Goulbourne CN, Zhang L, Hong C, Lin RR, *et al.* (2013) IDOL stimulates clathrin-independent endocytosis and multivesicular body-mediated lysosomal degradation of the low-density lipoprotein receptor. *Mol Cell Biol* **33**, 1503-1514.

Sorrentino V, Nelson JK, Maspero E, Marques AR, Scheer L, Polo S, *et al.* (2013) The LXR-IDOL axis defines a clathrin-, caveolae-, and dynamin-independent endocytic route for LDLR internalization and lysosomal degradation. *J Lipid Res* **54**, 2174-2184.

Physical Characteristics

Continued from page 1

Protein Sequence:

GPLGS***M***PAVASVPKELYLSSSLKDLNK
KTEVKPEKISTKSYVHSALKIFKTAEE
CRLDRDEERAYVLYMKYVTVYNLIKKRPD
FKQQQDYFHSILGPGNIKKAVEEAERLS
ESLKLRYEEAEVRKKLEEKDRQEEAQR
LQQKRQETGREDGGTLAKGSLENVLDSKDK
TQKSNGEKNEKCETKEKGAITAKELYTMMTD
KNISLIIMDARRMQDYQDSCILHSLSVPEE
AISPGVTASWIEAHLPDDSKDTWK
KRGNVEYVVLLDWFSSAKDLQIGTTL
RSLKDALFKWESKTVLRNEPLVLEGGYEN
WLLCYPQYTTNAKVTPPPRRQNEEVSISLD
FTYPSLEESIPSKPAAQTPPASIEVDEN
IELISGQNERMGPLNISTPVEPVAASKSD
VSPIIQPVPSIKNVPQIDRTKKPAVKLPEEH
RIKSESTNHEQQSPQSGKVIPDRSTKPVVF
SPTLMLTDEEKARIHAETALLMEKNKQEKEL
RERQQEEQKEKLRKEEQEQKAKKKQEAEE
NEITEKQQKAKEEMEKKESEQAKKEDKET
SAKRGKEITGVKRQSKSEHETSDAKKSVE
DRGKRCPTPEIQKKSTGDVPHTSVTGDSGS
GKPFKIKGGQPESGILRTGTFREDTD
DTERNKAQREPLTRARSEEMGRIVPGLPS
GWAKFLDPITGTFRYYHSPTNTVHMYPPE
MAPSSAPPSTPPTHKAKPQIPAERDREP
SKLKRSYSSPDITQAIQEEEKRKPTVTPTVN
RENKPTCYPKAEISRLSASQIRNLNPVFGGS
GPALTGLRNLGNTCYMNSILQCLCNAPHLA
DYFNRNCYQDDINRSNLLGHKGEVAEEFGI
IMKALWTGQYRYISPKDFKITIGKINDQF
AGYSQQDSQELLLFLMDGLHEDLNKAD
NRKRYKEENNDHLDDFKAAEHAWQKHKQL
NESIIVALFQGQFKSTVQCLTCHKKSRT
FEAFMYLSLPLASTSKCTLQDCLRLFSKEE
KLTDNNRFYCSHCRARRDSLKKIEIWKLP
PVLLVHLKRFSYDGRWKQKLQTSVDFPLEN
LDLSQYVIGPKNNLKKYNLFSVSNHYGGLDG
GHYTAYCKNAARQRWFKFDDHEVSDISVSS
VKSSAAYILFYTSLGPRVTDVAT

The residues underlined remain after cleavage and removal of the purification tag.
USP8 (regular text): Start ***bold italics*** (amino acid residues 1-1118)
Accession number: NP_005145

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