USP21 CD(196-565) [GST-tagged]

Deconjugating enzyme: Deubiquitylase

Alternate Names: USP23, Ubiquitin carboxyl terminal hydrolase 21, Ubiquitin carboxyl terminal hydrolase 23, Ubiquitin specific protease 21, Ubiquitin specific protease 23, Ubiquitin thioesterase 21, Ubiquitin thioesterase 23

Cat. No.	**64-0037-050**	Quantity:	50 µg
Lot. No.	**30396**	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 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 21 (USP21) is a member of the cysteine protease enzyme family and cloning of the gene was first described by Gong *et al.* (2000). USP21 cleaves ubiquitin polymers, and with reduced activity also targets the UBL ISG15 but not NEDD8 (Ye *et al.,* 2011). USP21 has been shown to be involved in the regulation of transcriptional initiation through the deubiquitylation of histone H2A as well as playing a role in the regulation of tumour necrosis factor α (TNFα) induced nuclear factor κβ (NF-κβ) activation by deubiquitylat-

Continued on page 2

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: ~67.5 kDa

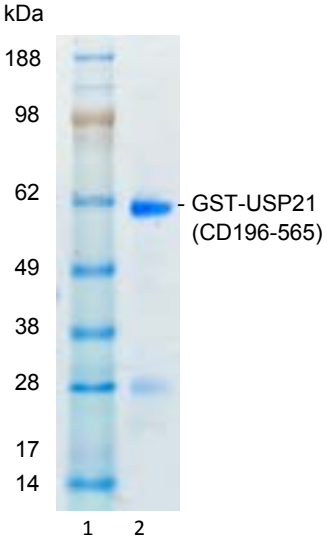
Purity: >79% by InstantBlue™ SDS-PAGE

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-USP21 CD(196-565)



Protein Identification:
Confirmed by mass spectrometry.

Deubiquitylase Enzyme Assay:
The activity of GST-USP21 CD(196-565) 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-USP21 CD(196-565) was compared confirming the deubiquitylating activity of GST-USP21 CD(196-565).

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Lot-specific COA version tracker: v1.0.0

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Background

Continued from page 1

ing receptor-interacting protein 1 (RIP1) (Nakagawa *et al.,* 2008; Xu *et al.,* 2010). Proteomic analyses also identified microtubule affinity-regulating (MARK) protein kinases and phosphatases as USP21 interactors, suggesting roles for USP21 in cell signalling (Li *et al.,* 2005). In a recent screen of 66 DUBs tagged with green fluorescent protein, USP21 was found to be unique by showing clear association with both centrosomes and microtubules (Urbe *et al.,* 2012).

References:

Gong L, Kamitani T, Millas S and Yeh ET (2000) Identification of a novel isopeptidase with dual specificity for ubiquitin- and NEDD8-conjugated proteins. *J Biol Chem* **275**, 14212-14216.

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.

Nakagawa T, Kajitani T, Togo S, Masuko N, Ohdan H, Hishikawa Y, *et al.* (2008) Deubiquitylation of histone H2A activates transcriptional initiation via trans-histone cross-talk with H3K4 di- and trimethylation. *Genes Dev* **22**, 37-49.

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

Urbe S, Liu H, Hayes SD, Heride C, Rigden DJ and Clague MJ (2012) Systematic survey of deubiquitinase localization identifies USP21 as a regulator of centrosome- and microtubule-associated functions. *Mol Biol Cell* **23**, 1095-1103.

Xu G, Tan X, Wang H, Sun W, Shi Y, Burlingame S, *et al.* (2010) Ubiquitin-specific peptidase 21 inhibits tumor necrosis factor alpha-induced nuclear factor kappaB activation via binding to and deubiquitinating receptor-interacting protein 1. *J Biol Chem* **285**, 969-978.

Ye Y, Akutsu M, Reyes-Turcu F, Enchev RI, Wilkinson KD and Komander D (2011) Polyubiquitin binding and cross-reactivity in the USP domain deubiquitinase USP21. *EMBO Rep* **12**, 350-357.

Physical Characteristics

Continued from page 1

Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEG
DKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYI
ADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAY
SKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRI
EAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKS
DS*D*DDKMAHHTLLLGSGHVGLRNLGNTCFLNAVLQCL
SSTRPLRDFCLRRDFRQEVPGGGRAQELTEAFADVIG
ALWHPDSCEAVNPTRFRAVFQKYVPSFSGYSQQDAQ
EFLKLLMERLHLEINRRGRRAPPILANGPVPSPPRRGG
ALLEEPELSDDDRANLMWKRYLEREDSKIVDLFVGQL
KSCLKCQACGYRSTTFEVFCDLSLPIPKKGFAGGKVSLR
DCFNLFTKEEELESENAPVCDRCRQKTRSTKKLTVQRF
PRILVLHLNRFSASRGSIKKSSVGVDFPLQRLSLGDFAS
DKAGSPVYQLYALCNHSGSVHYGHYTALCRCQTGWH
VYNDSRVSPVSENQVASSEGYVLFYQLMQEPPRCL

Tag (**bold text**): N-terminal GST
USP21 (regular text): Start ***bold italics*** (amino acid residues 196-565)
Accession number: NP_001014443

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Lot-specific COA version tracker: v1.0.0