

USP28 [GST-tagged]

Deconjugating enzyme: Deubiquitylase

Alternate Names: EC 3.1.2.15, KIAA1515

Cat. No. **64-0020-050**
Lot. No. **30019**

Quantity: **50 µg**
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 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 carboxyl-terminal hydrolase 28 (Ubiquitin Specific Protease 28; USP28) is a member of the cysteine protease enzyme family and cloning of the human gene was first described by Nagase *et al.* (2000). USP28 shares 51.4% identity with USP25 and is highly expressed in the heart and skeletal muscle (Valero *et al.*, 2001). Both USP28 and Checkpoint kinase Chk2 are required for DNA-damage-induced apoptosis, and they accomplish this partly through regulation of the p53 induction of proapoptotic genes. USP28 is required to stabilize Chk2, p53 binding protein 53BP1 and claspin in response to DNA double

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Physical Characteristics

Species: human

Protein Sequence: Please see page 2

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

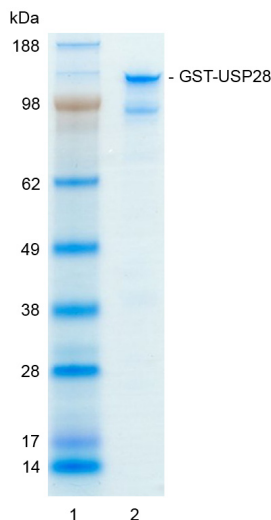
Molecular Weight: ~149 kDa

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-USP28



Protein Identification:
Confirmed by mass spectrometry.

Deubiquitylase Enzyme Assay:
The activity of GST-USP28 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-USP28 was compared confirming the deubiquitylating activity of GST-USP28.



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Lot-specific COA version tracker: v1.0.0

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Background

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strand breaks (Zhang *et al.*, 2006). USP28 stabilizes the MYC proto oncogene by antagonising the activity of the SCF-FBW7 ubiquitin ligase complex. MYC is a regulator of cell growth, proliferation and apoptosis, and enhanced levels are thought to contribute to the genesis of human tumours such as breast and colon carcinomas. Inhibition of USP28 may be a pharmacologically feasible approach to interfere with MYC function in human tumours (Popov *et al.*, 2007).

References:

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

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.

Popov N, Wanzel M, Madiredjo M, Zhang D, Beijersbergen R, Bernards R, Moll R, Elledge SJ, Eilers M (2007) The ubiquitin-specific protease USP28 is required for MYC stability. *Nat Cell Biol* 9, 765-774.

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

Valero R, Bayes M, Francisca Sanchez-Font M, Gonzalez-Angulo O, Gonzalez-Duarte R, Marfany G (2001) Characterization of alternatively spliced products and tissue-specific isoforms of USP28 and USP25. *Genome Biol* 2, RESEARCH0043.

Zhang D, Zaugg K, Mak TW, Elledge SJ (2006) A role for the deubiquitinating enzyme USP28 in control of the DNA-damage response. *Cell* 126, 529-542.

Physical Characteristics

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Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYYIDGD
VKLTQSMAIRYIADKHNMLGGCPKERAEISM
LEGAVLDIRYGVSR IAYS KDFETLKVDFL
SKLPEMLKMFEDRLCHKTYLNGDHVTHPD
FMLYDALDVVL YMDPMCLDAFPKLVCFK
KRIEAI PQIDKYLKSSKYIAWPLQGQWATFG
GGDHPPKSDLEVL**FQGP**LP**GSMTAELQOD**
DAAGAADGHGSSCQMLLNQLREITGIQDPS
FLHEALKASNGDITQAVSLLTDERVKEP
SQDVTATEPSEVEGSAANKEVLAKVIDLTH
DNKDDLQAAIALSLLLESPKIQADGRDLNRM
HEATS AETKRSKRKRCEVWGENPNPNDR
RVDGWVPLKNGVNTCWFSAVIQSLFQLPE
FRRLVLSYSLPQNVLENCRSHTEKRNIMF
MQELQYLFALMMGSRKRVDP SAALDLLKGA
FRSSEEQQDVSEFTHKLLDWLEDAFQLAVN
VNSPRNKSENPMVQLFYGTFLTEGVREGK
PFCNNETFGQYPLQVNGYRNLDECLEGAM
VEGDVELLPSDHSVYKYGQERWFTKLP
PVLTFELSRFEFNQSLGQPEKIH NKLEF
PQIIYMDRYMYRSKELIRNKRECI RKL
KEEIKILQOKLERYVYKYGSGPARFPLPDM
LKYVIEFASTKPASECPPESDTHMTLPLSS
VHCSVSDQTSKESTSTESSSQDVESTFSS
PEDSLPKSKPLTSSRSMEMPSPQAPART
VTDEEINFVKTC LQRWRSEIEQDIQDLKT
CIASTTQTIEQMYCDPLLRQVPYRLHAV
LVHEGQANAGHYWAYIYNQPRQSWLKYNDIS
VTSSWEEVERDSYGGLRNVSA YCLMY
INDKLPYFNAEAAPTESDQMS EVEALS
VELKHYIQEDNWRFEQVEVEWEEEQSCKIP
QMESSTNSSSQDYSTSQEPSVASSHGVR
CLSEHAVIVKEQTAQAIANTARAYEKS
GVEAALS EVMLS PAMQGVILAI AKAR
QTFDRDGSEAGLIKAFHEEYSRLYLAKETPT
SHSDPRLQHVLVYFFQNEAPKR VVERTLLEQ
FADKNLSYDERSISIMKVAQAKLKEIGPD
DMNMEEYKKWHEDYS LFRKVS VYLLTGLE
LYQKGYQEALS YLVYAYQSNAALLMKG
PRRGVKESVIALYRRKCLLELNAKAASLFET
NDDHSVTEGINVMNELIIPC IHLIINNDISKD
DLDAIEVMRNHWCSYLGQDIAENLQLCLGEFL
PRLLDPSAEIIVLKEPPTIRPNSPYDLC SR
FAVMESI QGVSTVTVK

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVL**FQ**▼GP)
USP28 (regular text): Start **bold italics** (amino acid residues 1-1077)
Accession number: NP_065937



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