

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active USP35 [390 – 978]

Enzyme description:- USP35 [390 - 978]

Clone number:- DU 67487

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 93, 085.22 daltons

Average Mass 93, 144.23 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.17

Purity:- 80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1 mM EGTA, 10 mM DTT

Storage temperature:- -70 °C

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Clone Data Sheet

USP35 [390 - 978]

<u>Protein</u>	USP35 [390 - 978]
<u>Clone number</u>	DU 67487
<u>Species</u>	Human
<u>Accession number</u>	Q9P2H5-1
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDENLYFQGGSLYEPVMEAIKDLHVPN EDRIKQLLGQDAWTSQKSELAGFYPR LMAKSDTGKIGLINLGNTCYVNS ILQALFMASDFRHCVLRRLTENNSQPLMTKLQWLFGFLEHSQRPAISPEN FLSASWTPWFSPGTQODCSEYLKYL LDR LH EEEKTGTRICQK LKQSSSP SPPEEPPAPSSTSVEKMFGGKIVTRICCLCLNVSSREEAFTDLSLAFP PPERCRRRRLGSMRPTEDITARELPPPTSAQGPGRVGP RRQRKHCITE DTPPTSLYIEGLDSKEAGGQSSQEERIEREEEGKEERTEKEEVGEEEEES TRGEGEREKEEEVEEEEEKVEKETEKEAEQEKEEDSLGAGTHPDAAIPS GERTCGSEGSRSVLDLVNYFLSPEKLT AENRYYC ESCASLQDAEKVVEL SQGPCYLILTL LRF SFDLRTMRRRKILDDVSIPLLLRLPLAGGRGQAYD LCSVVVHSGVSSSESGHYCYAREGAARPAASLGTADRPEPENQWYLFND TRVSFSSFESVSNVTSFFPKDTAYVLFYRQRPREGPEAELGSSRVRTEP TLHKDLMEAI SKDNILYLQEQEKEARSRAAYISA</p>
<u>Native sequence</u>	Amino acids L390 – A978 (end residue is F1018) of human USP35. Residue L230 of the fusion protein is equivalent to L390 of the native enzyme. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	rTEV (<u>ENLYFQG</u>) residues 221 - 227
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> 1 sites of pGEX TEV

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Nucleotide
Sequence of insert

ggatccCTGTACGAGCCCGTGATGGAAGCCATCAAGGACCTGCACGTGC
CCAACGAGGACCGGATCAAGCAACTGCTGGGCCAGGACGCCTGGACCAG
CCAGAAGTCCGAGCTGGCCGGCTTCTACCCCAGGCTGATGGCCAAGAGC
GACACCGGCAAGATCGGCCTGATCAACCTGGGCAATACCTGCTACGTGA
ACAGCATCCTGCAGGCCCTGTTTCATGGCCAGCGACTTCCGGCACTGCGT
GCTGCGGCTGACCGAGAACAACAGCCAGCCCCTGATGACCAAGCTGCAG
TGGCTGTTTCGGCTTTCTGGAACACTCCCAGAGGCCCGCTATCAGCCCCG
AGAACTTCCTGAGCGCCAGCTGGACCCCCTGGTTCAGCCCTGGCACCCA
GCAGGACTGCAGCGAGTACCTGAAGTATCTGCTGGACCGGCTGCACGAG
GAAGAGAAAACCGGCACCCGGATCTGCCAGAAGCTGAAGCAGAGCAGCA
GCCCCAGCCCCCTGAGGAACCTCCTGCCCAAGCAGCACCAGCGTGGGA
AAAGATGTTTCGGCGGAAAGATCGTGACCAGAATCTGCTGCCTGTGCTGT
CTGAACGTGTCCAGCCGGGAAGAGGCCTTCACCGACCTGAGCCTGGCCT
TCCCACCCCCGAGAGATGCAGAAGGCGGAGACTGGGCAGCGTGATGCG
GCCCACCGAGGACATCACCGCCAGAGAGCTGCCTCCCCCAACAAGCGCC
CAGGGACCTGGCAGAGTGGGCCCCAGACGGCAGCGGAAGCACTGCATCA
CCGAGGATACCCCCCCCACCAGCCTGTACATCGAGGGCCTGGACAGCAA
AGAGGCTGGCGGCCAGAGCAGCCAGGAAGAGAGAATCGAGCGCGAGGAA
GAGGGCAAAGAGGAACGCACCGAGAAAGAGGAAGTCGGCGAGGAAGAAG
AGAGCACCAGAGGCGAGGGCGAGCGCGAAAAAGAGGAAGAAGTCGAAGA
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TACTACTGCGAGAGCTGCGCCAGCCTGCAGGACGCCGAGAAGGTGGTGG
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CTTCGACCTGCGGACCATGCGGCGGAGAAAGATTCTGGACGACGTGTCC
ATCCCCCTGCTGCTGAGGCTGCCTCTGGCTGGCGGAAGAGGCCAGGCCT
ACGACCTGTGCAGCGTGGTGGTGCACAGCGGCGTGTCCAGCGAGAGCGG
CCACTACTACTGCTACGCCAGAGAGGGCGCTGCCAGACCAGCCGCCTCT
CTGGGCACAGCCGATAGACCCGAGCCCAGAAACCAGTGGTATCTGTTCA
ACGACACCAGAGTGTCTTCAGCAGCTTCGAGAGCGTGTCCAACGTGAC
CTCATTCTTCCCAAAGGACACCGCCTACGTGCTGTTCTACCGGCAGCGG
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AGCCCACCTGCACAAGGACCTGATGGAAGCTATCTCCAAGGACAACAT
CCTGTATCTGCAGGAACAGGAAAAAGAGGCCAGAAGCAGAGCCGCCTAC
ATCAGCGCctgagcggccgc