

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of USP35 C450A [1 - 1018]

<u>Enzyme description:-</u>	USP35 C450A [1 – 1018]
<u>Clone number:-</u>	DU 25786
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	GSH Sepharose

Calculated molecular mass:-

Monoisotopic	140, 107.97 daltons
Average Mass	140, 197.13 daltons
[cysteines reduced, methionines have not been oxidised]	

Theoretical pI:- 5.48

Purity:- >80 %

Activation protocol:-	Constitutively active
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Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSE

Storage temperature:- -70 °C

Assay buffer:-

50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM MgAc

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Clone Data Sheet

USP35 C450A [1 - 1018]

Protein USP35 C450A [1 - 1018]

Clone number DU 25786

Species Human

Accession number Q9P2H5

Tags N-terminal GST

**Baculovirus
expressed protein**

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAETISMLE
GAVLDIRYGVSRIAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY
LKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFGQPLGSM**KILEAVV**
TSSYPVSVKQGLVRRVLEAARQPLEREQCLALLALGARLYVGGAEELP
RRVGCQLLHVAGRHPDVFAEFFSARRVLRLQLGGAGPPGPRALACVQ
LGLQLLPEGPAADEVFALLRREVLRTVCERP GPAACAQVARLLARHPR
CVPDGPHRLLFQQLVRC LGRFCPAEGEEGAVEFLEQAQOVSGLLAQ
LWRAQPAAILPCLKELFAVISCAEEPPSSALASVVQHLPLELMDGVV
RNLSNDDSVTDSQMLTAISR MIDVSWPLGKNIDKWI IALLKGLAAVK
KFSILIEVSLTKIEKVFSKLLYP IVRGAALSVLKYMLLTFQHSHEAFH
LLLPHIPPMVASLVKEDSNSGTSCLEQLAELVHCMVFRFPGF PDLYEP
VMEAIKDLHVPNEDRIKQLLGQDAWTSQKSELAGFYPR LMAKSDTGKI
GLINLGNTAYVNSILQALFMASDFRHCVLRLTENNSQPLMTKLQWLFG
FLEHSQRPAISPENFLSASWTPWFSPGTQQDCSEYLKYLLDRLHEEEK
TGTRICQKLKQSSSPSPPEEPPAPSSTSVEKMF GGKIVTRICCLCCLN
VSSREEAFTDLSLAFPPPERCRRRLGSMRPTEDITARELP PPTSAQ
GPGRVGPRRQRKHCITEDTPPTS LYEGLDSKEAGQSSQEERIEREE
EGKEERTEKEEVGEEEESTRGEGEREKEEEVEEEEEKVEKETEKAEQ
EKEEDSLGAGTHPDAAIPSGERTCGSEGSRSVLDLVNYFLSPEKLTAE
NRYYCESCASLQDAEKVVELSQGPCYLILTL LRF SFDLRTMRRRKILD
DVSIPLLLRLPLAGGRGQAYDLCSVVVHSGVSS ESGHYCYAREGAAR
PAASLGTADRPEPENQWYLFNDTRVSFSSFESVSNVTSFFPKDTAYVL
FYRQRPREGPEAELGSSRV RTEPTLHKDLMEAI SKDNILYLQEQEKEA
RSRAAYISALPTS PHWGRGFDEDKDEDEGSPGGCNPAGNGGDFHRLV
F

Native sequence Amino acids M1 – F1018 (end) of human USP35. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220. The protein has a C450A mutation. Residue C450 is equivalent to residue A681 of the fusion protein.

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Protease cleavage PreScission site (LEVLFQGP) residues 221 – 228

Cloning sites *Bam*H1 and *Not*I sites of pFastBac GST 6P1

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Nucleotide sequence of insert

ggatccATGGACAAGATCTTGGAGGCGGTGGTGACGTCGTCATACCCG
GTCAGCGTGAAGCAGGGGCTGGTTGCGCGCGTGCTGGAGGCGGCGCGG
CAGCCGCTGGAGCGTGAGCAGTGCCTGGCGCTGCTGGCGCTGGGCGCG
CGCCTCTACGTGGGCGGCGCGGAGGAGCTGCCGCGCCGCGTGGGCTGC
CAGCTGCTGCACGTGGCCGGCCGCCACCAACCCGACGTCTTCGCCGAG
TTCTTCAGCGCGCGTTCGCGTGCTGCGCCTGCTGCAGGGTGGCGCCGGC
CCCCGGGCCCCCGCGCGCTCGCCTGCGTGACGCTGGGTCTGCAGCTG
CTGCCCAGGGGGCCTGCGGCCGACGAGGTGTTGCGCTGCTGCGGCGC
GAGGTGCTGCGCACCGTGTGCGAGCGCCCGGGCCCCGCGGCCTGCGCG
CAGGTGGCACGGCTGCTGGCTCGCCACCCGCGCTGTGTGCCCGACGGA
CCCCACCGCCTGCTCTTCTGCCAGCAGCTGGTGCCTTGCCTCGGCCGC
TTCCGCTGCCCAGCCGAAGGCGAGGAGGGCGCCGTGGAGTTCCCTAGAG
CAGGCCCAGCAGGTGAGCGGGCTCCTGGCGCAGCTGTGGCGCGCACAG
CCCGCCGCCATCCTGCCCTGCCTCAAAGAGCTGTTTCGAGTCATCTCC
TGCGCAGAGGAGGAGCCACCATCTAGCGCCCTGGCCAGCGTGGTCCAG
CACCTCCCATTGGAGCTCATGGATGGTGTGTCCGGAACCTCAGCAAT
GATGACAGTGTGACAGACTCGCAGATGCTGACTGCCATTAGCAGGATG
ATTGACTGGGTGTCCTGGCCCCCTGGGGAAGAATATTGACAAGTGGATC
ATTGCACTGCTGAAGGGCCTGGCTGCTGTTAAGAAGTTCAGCATCTTG
ATCGAGGTTTCGCTCACCAAAATTGAGAAGGTTTTCTCTAAGCTGCTG
TACCCCATCGTCCGGGGAGCTGCCTTGTCTGTGCTCAAGTACATGCTC
CTGACCTTCCAGCACTCCCACGAAGCCTTCCACCTGCTCCTCCCTCAC
ATCCCCCCCATGGTGGCCTCTCTGGTCAAGGAGGACTCGAACTCGGGG
ACCAGCTGCCTGGAGCAGCTGGCGGAGCTGGTCCACTGCATGGTGTTC
CGGTTCCTGGGCTTCCCGGATCTGTATGAGCCTGTCATGGAGGCCATC
AAGGACCTCCATGTTCCCAATGAGGACCGCATCAAGCAGCTGCTGGGG
CAGGATGCCTGGACTTCGCAGAAGAGCGAGCTGGCGGGTTTTCTATCCC
CGGCTCATGGCCAAGTCAGACACGGGCAAGATTGGTCTCATCAACCTG
GGCAACACAGCCTATGTCAACAGCATCCTTCAGGCCTTATTCATGGCG
TCTGACTTCAGACATTGTGTGCTCCGCTTGACTGAGAACTCACTACAG
CCCCTGATGACCAAGCTGCAGTGGCTCTTTGGCTTCCTAGAACACAGC
CAGCGGCCTGCCATTTCCCCAGAGAACTTCCTCTCCGCATCCTGGACG
CCCTGGTTCAGCCCTGGCACCCAGCAGGACTGCTCGGAGTATCTGAAG
TACCTGCTGGATCGGCTGCACGAAGAGGAGAAAACGGGCACAAGGATC
TGCCAGAACTCAAGCAGTCCAGCTCGCCCTCTCCGCCCCGAGGAGCCC
CCGGCCCCAAGTTCAACCTCTGTGGAATAATGTTTGGAGGCAAGATA
GTGACTCGGATCTGCTGTCTCTGCTGCCTCAACGTCTCCTCCCGGGAG
GAGGCCTTCACGGACCTCTCTCTCGCCTTCCCTCCTCCTGAGCGCTGT
CGCCGCCGCGCCTGGGCTCTGTGATGCGCCCCACAGAAGACATCACA
GCCCCGGGAGTTGCCCCCACCAACCAGTGACAGAGGGCCAGGCAGGGTG
GGTCTCGGAGGCAAAGGAAACACTGCATCACAGAGGACACCCCCCCC
ACCAGCCTGTACATCGAAGGCCTGGACTCCAAGGAAGCTGGTGGGCAG
AGCAGTCAGGAGGAAAGGATAGAGAGGGAGGAAGAAGGGAAGGAGGAG
AGAACGGAGAAGGAAGAAGTGGGGGAGGAGGAGGAAGCACCAGAGGG
GAAGGAGAGAGGGAGAAAAGAGGAGGAGGTGGAAGAGGAAGAAGAGAAG
GTGGAGAAGGAGACAGAAAAGGAGGCTGAGCAGGAAAAGGAAGAAGAC
AGCCTGGGAGCGGGGACCCACCCGGATGCTGCCATCCCCTCCGGGGAG

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CGGACATGTGGCTCTGAGGGCTCCCGCTCCGTCCTGGACCTGGTTAAC
TACTTCCTGTCCCCGAGAAGCTGACAGCAGAAAACCGTACTACTGC
GAGTCGTGTGCCTCCCTGCAGGATGCCGAGAAGGTGGTGGAGCTGAGC
CAAGGGCCGTGCTACCTCATCCTCACACTGCTGCGCTTCTCTTTCGAC
CTGCGCACCATGCGGCGCCGCAAGATCCTGGATGACGTCTCCATCCCC
CTGCTGCTCCGCCTGCCACTGGCTGGTGGCCGTGGCCAGGCCTATGAC
CTCTGCAGTGTGGTGGTGCACCTCTGGAGTGTCTTCGGAGAGTGGTCAC
TACTACTGCTATGCCCCGTGAGGGCGCTGCCCCGCCCTGCCGCTTCTCTG
GGAAGTCCGATAGGCCAGAGCCCGAGAACCAGTGGTACCTGTTCAAT
GACACTCGGGTGTCTTCTCTTCCCTTCGAATCTGTCAGCAACGTCACC
TCCTTCTTCCCTAAGGACACAGCCTATGTGCTGTTTTACCGGCAGCGG
CCCAGGGAGGGGCCCCGAGGCTGAGTTGGGCTCTTCTAGAGTCCGGACA
GAGCCACCCCTGCACAAGGACTTGATGGAAGCCATTTCCAAAGACAAC
ATCCTTTACCTACAGGAGCAGGAGAAGGAGGCCCGGAGCAGGGCGGCC
TACATCTCTGCACTCCCCACATCTCCGCACTGGGGGAGGGGCTTTGAT
GAAGACAAGGATGAGGATGAAGGCTCTCCAGGGGGCTGCAATCCTGCA
GGTGGCAATGGTGGTGACTTCCACAGACTGGTCTTCTaagcggccgc