

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active USP19 [1 - 1318]

<u>Enzyme description:-</u>	USP19 [1 - 1318]
<u>Clone number:-</u>	DU 37789
<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 172,739.03 daltons
Average Mass 172,848.37 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.84

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

USP19 [1 - 1318]

Protein USP19 [1 - 1318]

Clone number DU 37789

Species Human

Accession number O94966

Tags N-terminal GST

**Baculovirus
expressed protein**

MSPILGYWKIKGLVQPTRLLLEYLEEKYEHLIERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLQSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSR IAYSKDFETLKVDFLSKLP EMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFOGPLGSPEFMSGGAS
ATGPRRGPPGLEDTTSKKKQKDRANQESKGDPRKETGSRVVAQAGLE
PLASGDP SASASHAAGITGSRHRTRLFFPSSSGSASTPQEEQTKEGAC
EDPHDLLATPTPELLLDWRQSAEEVIVKLRVGVGPLQLEDVDAFTDT
DCVVRFAGGQQWGGVFYAEIKSSCAKVQTRKGSLLHLTLPKKVPMLTW
PSLLVEADEQLCIPPLNSQTCLLGSEENLAPLAGEKAVPPGNDPVSPA
MVRSRNP GKDDCAKEEMAVAADAATLVDEPESMVNLA FVKND SYEKGP
DSVVVHVYVKEICRDTSRVLFREQDFTLIFQTRDGNFLRLHPGCGPHT
TFRWQVKLRNLI EPEQCTFCFTASRIDICLRKRQSORWGGLEAPAARV
GGAKVAVPTGPTPLDSTPPGGAPHPLTGQEEARAVEKDKSARSED TG
LDSVATRTPMEHVTPKPEHLASPKPTCMVPPMPHSPVSGDSVEEEEE
EEKKVCLPGFTGLVNLGNTCFMNSVIQSLSNTR ELRDFHDRSFEAEI
NYNNPLGTGGRLAIGFAVLLRALWKGTHHAFQPSK LKAI VASKASQFT
GYAQHDAQEFMAFLLDGLHEDLNRIQNKPYTETVDS DGRPDEVVAEEA
WQRHKMRNDSFIVDLFQGOYKSKLVCPVCAKVSITFD PFLYLPVPLPQ
KQKVL PVFYFAREPHSKPIKFLVSVSKENSTASEVLD SLSQSVHVKPE
NLRLAEV IKNRFHRVFLPSHSLDTVSPSDTLLCFELLSSELAKERVVV
LEVQQR PQVSPVISKCAACQRKQOSEDEKLKRCTR CYRVGYCNQLCQ
KTHWPDHKGLCRPENIGYPFLVSVPASRLTYARLAQLLEGYARYSVSV
FQPPFQPRMALESQSPGCTLLSTGSLEAGDSERDPIQPPELQLVTP
MAEGDTGLPRVWAAPDRGPVSTSGISSEMLASGPIEVGSLPAGERVS
RPEAAVPGYQHPSEAMNAHTPQFFIYKIDSSNREORLEDKGDTPLELG
DDCSLALVWRNNERLQEFVLVASK ELECAEDPGSAGEAARAGHFTLDQ
CLNLFTRPEVLAPEEAWYCPQCKQHREASKQLLLWRLPNVLI VQLKRF
SFRSFIWRDKINDLVEFPVRNLDLSKFCIGQKEEQ LPSYDLYAVINHY
GGMIGGHYTACARLPNDRSSQRSVDVWRLFDDSTVTTVDESQVVTRYA
YVLFYRRRNSPVERPPRAGHSEHHPDLGPAAEAAAASQASRIWQELEAE
EFPVPEGSGPLGPWGPQDWVGPLPRGPTTPDEGCLRYFVLGTVAALVA
LVLNVFYPLVSQSRWR

Native sequence Amino acids M1 – R1318 (end residue) of human USP19.

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Residue M235 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 - 220.

Protease cleavage PreScission (LEVLFQGP) residues 221 - 228

Cloning sites *EcoRI* and *SalI* sites of pFastBac Dual.

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Nucleotide sequence of insert

gaattcATGTCTGGCGGGCCAGTGCCACAGGCCCAAGGAGAGGGCCCC
CAGGACTGGAGGACACCCTAGTAAGAAGAAGCAGAAGGATCGAGCAAA
CCAGGAGAGCAAGGATGGAGATCCTAGGAAAGAGACAGGGTCTCGATAT
GTTGCCCAGGCTGGTCTTGAACCTCTGGCCTCAGGTGATCCTTCTGCCT
CAGCCTCCCATGCAGCTGGGATCACAGGCTCACGCCACCGTACCCGGCT
GTTCTTTCCTTCATCGTCAGGGTCAGCATCCACTCCTCAAGAGGAGCAG
ACCAAAGAGGGAGCTTGTGAAGACCCTCATGATCTCTTGGCTACTCCA
CTCCAGAGTTGTTGCTCGATTGGAGGCAGAGTGCAGAAGAGGTGATTGT
CAAGCTTCGTGTGGGAGTAGGTCCCCGCAGCTGGAGGATGTAGATGCT
GCTTTCACAGATACAGACTGTGTGGTGC GGTTTTGCAGGTGGTCAGCAGT
GGGGTGGTGTCTTCTATGCTGAGATAAAAAGCTCTTGTGCTAAAGTGCA
AACCCGCAAGGGCAGTCTCCTGCACCTGACACTGCCCAAAAAGGTGCCT
ATGCTCACGTGGCCCTCCCTCCTGGTTGAGGCTGATGAACAGCTTTGCA
TACCACCGCTGAACTCCCAAACCTGCCTCCTGGGCTCAGAGGAGAATTT
AGCCCCTTTGGCAGGAGAGAAAGCAGTGCCTCCCGGGAATGACCCAGTC
TCTCCAGCCATGGTCCGGAGCAGAAAACCCTGGGAAAAGATGACTGTGCCA
AGGAGGAGATGGCAGTGGCAGCAGATGCTGCAACCTTGGTGGATGAGCC
CGAGTCGATGGTGAACCTGGCGTTTGTCAAGAATGACTCGTATGAGAAG
GGCCCGGATTCAGTGGTGGTGCACGTGTACGTGAAGGAGATCTGCAGGG
ACACCTCAAGAGTACTTTTCCGTGAGCAGGACTTCACACTCATCTTCCA
GACCAGGGATGAAAACCTCCTGAGGCTGCACCCGGGCTGTGGGCCCCAC
ACCACCTTCCGTTGGCAGGTGAAGCTCAGGAATCTGATTGAGCCAGAGC
AGTGCACCTTCTGTTTACGGCTTCTCGCATCGACATCTGCCTTCGTAA
GAGGCAGAGTCAGCGCTGGGGGGGCTGGAGGCCCCGGCTGCACGAGTG
GGTGGTGCAAAGGTTGCCGTGCCGACAGGTCCAACCCCTCTGGATTCAA
CCCCACCAGGAGGTGCTCCCCACCCCTGACAGGCCAGGAGGAGGCCCG
GGCTGTGGAGAAGGATAAATCCAAGGCACGATCTGAGGACACAGGGCTA
GACAGTGTGGCAACCCGCACACCCATGGAGCATGTAACCCCAAAGCCAG
AGACACACCTGGCCTCGCCAAAGCCTACATGCATGGTGCCTCCCATGCC
CCACAGCCCAGTTAGTGGAGACAGCGTGGAGGAGGAGGAAGAGGAAGAG
AAGAAGGTGTGTCTGCCAGGCTTCACTGGCCTTGTCAATTTAGGCAACA
CCTGCTTCATGAACAGCGTCATTCAGTCTCTGTCCAACACTCGGGAAC
CCGGGACTTCTTCCATGACCGCTCCTTTGAGGCTGAGATCAACTACAAC
AACCCACTAGGGACTGGTGGGCGTCTGGCCATTGGCTTTGCCGTGCTGC
TTCGGGCGCTGTGGAAGGGCACCCACCATGCCTTCCAGCCTTCCAAGTT
GAAGGCCATTGTGGCGAGTAAGGCCAGCCAGTTCACAGGCTATGCACAG
CATGATGCCCAGGAGTTCATGGCTTTCCTGCTGGATGGGCTGCACGAGG
ACCTGAATCGCATTGAGAACAAGCCCTACACAGAGACCGTGGATTGAGA
TGGGCGGCCGATGAGGTGGTAGCTGAGGAAGCATGGCAGCGGCACAAG
ATGAGGAATGACTCTTTCATCGTGGACCTATTTGAGGGGAGTACAAGT
CGAAGCTGGTGTGCCCTGTGTGTGCCAAGGTCTCCATCACTTTTGACCC
GTTTCTTTATCTGCCGGTGCCCTTGCACAAAAGCAAAAGGTTCTCCCT
GTCTTTTATTTTGCCGAGAGCCCCACAGCAAGCCATCAAGTTCTCTGG
TGAGCGTCAGCAAGGAGAACTCCACTGCGAGCGAAGTATTGGACTCCCT
CTCTCAGAGTGTTTCATGTGAAGCCTGAGAACCCTGCGTTTGGCGGAGGTA
ATTAAGAATCGTTTTTCATCGTGTGTTCCCTACCCTCCCCTCACTGGACA
CTGTGTCCCCATCTGATACGCTCCTCTGCTTTGAGCTGCTATCCTCAGA
GTTGGCTAAGGAGCGGGTAGTGGTGTAGAGGTGCAACAGCGCCCCCAG
GTGCCCAGCGTCCCCATCTCCAAGTGTGCAGCCTGCCAGCGGAAGCAAC
AGTCGGAGGATGAAAAGCTGAAGCGCTGTACCCGGTGTACCGTGTGGG
CTACTGCAACCAGCTCTGCCAGAAAACCCACTGGCCTGACCACAAGGGC
CTCTGCCGACCTGAGAACATTGGCTACCCCTTCCCTGGTCAGTGTACCTG
CCTCACGCCTCACTTATGCCCCGCTCGCTCAGTTGCTAGAGGGCTATGC
CCGGTACTCTGTGAGTGTATTCCAGCCACCCTTTCAGCCAGGCCGCATG
GCCTTGGAGTCTCAGAGCCCTGGCTGCACCACACTGCTCTCCACAGGTT
CCCTGGAGGCTGGGGACAGCGAGAGAGACCCCACTCAGCCACAGGACT
CCAGCTGGTGAACCCCTATGGCTGAGGGGGACACAGGGCTTCCCCGGGTG
TGGGCAGCCCCTGACCGGGGTCTGTGCCAGCACCAGTGGAATTTCTT

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CTGAGATGCTGGCCAGTGGGCCCATTGAGGTTGGCTCCTTGCCAGCTGG
CGAGAGGGTGTCCCGACCCGAAGCTGCTGTGCCTGGGTACCAGCATCCA
AGTGAAGCTATGAATGCCACACACCCAGTTCTTCATCTATAAAATG
ATTCATCCAACCGAGAGCAGCGGCTAGAGGACAAAGGAGACACCCCACT
GGAGCTGGGTGACGACTGTAGCCTGGCTCTCGTCTGGCGGAACAATGAG
CGCTTGCAGGAGTTTGTGTTGGTAGCCTCCAAGGAGCTGGAATGTGCTG
AGGATCCAGGCTCTGCCGGTGAGGCTGCCCGGGCCGCACTTCACCCT
GGACCAGTGCCTCAACCTCTTCACACGGCCTGAGGTGCTGGCACCCGAG
GAGGCCTGGTACTGCCACAGTGCAAACAGCACCGTGAGGCCTCCAAGC
AGCTGTTGCTATGGCGCCTGCCAAATGTTCTCATCGTGCAGCTCAAGCG
CTTCTCCTTTCGTAGTTTTATCTGGCGTGACAAGATCAATGACTTGGTG
GAGTTCCTGTTAGGAACCTGGACCTGAGCAAGTTCTGCATTGGTCAGA
AAGAGGAGCAGCTGCCCAGCTACGATCTATATGCTGTCATCAACCACTA
TGGAGGCATGATTGGTGGCCACTACACTGCCTGTGCACGCCTGCCCAAT
GATCGTAGCAGTCAGCGCAGTGACGTGGGCTGGCGCTTGTTTTGATGACA
GCACAGTGACAACGGTAGACGAGAGCCAGGTTGTGACGCGTTATGCCTA
TGTA CTCTTCTACCGCCGGCGGAACTCTCCTGTGGAGAGGCCCCCCAGG
GCAGGTC ACTCTGAGCACCACCCAGACCTAGGCCCTGCAGCTGAGGCTG
CTGCCAGCCAGGCTTCCCGGATTTGGCAGGAGCTGGAGGCTGAGGAGGA
GCCGGTGCCTGAGGGGTCTGGGCCCTGGGTCCCTGGGGGCCCAAGAC
TGGGTGGGCCCTACCACGTGGCCCTACCACACCAGATGAGGGCTGCC
TCCGGTACTTTGTCTGGGCACCGTGGCGGCTTTGGTGGCCCTCGTGCT
CAACGTGTTCTATCCTCTGGTATCCAGAGTCGCTGGAGAtgagtcgac