

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active USP15 [1 - 952]

<u>Enzyme description:-</u>	USP15 [1 - 952]
<u>Clone number:-</u>	DU 37753
<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 137,012.41 daltons
Average Mass 137,099.92 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.12

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

USP15 [1 - 952]

Protein USP15 [1 - 952]
Clone number DU 37753
Species Human
Accession number Q9Y4E8-2
Tags N-terminal GST

**Baculovirus
expressed protein**

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNK
KFELGLEFPNLPYYIDGDVKLTSMAIIRYIADKHNMLGGCPKE
RAEISMLEGAVLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKM
FEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKL
VCFKKRIEAIPOIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSD
LEVLFGQPLGSPGIPGSTRAAAMAEGGAADLDTQRSDIATLLKT
SLRKGDTWYLVDSRWFQWKKYVGFDSWDKYQMGDQNVYPGPID
NSGLLKDGDAQSLKEHLIDELDYILLPTEGWNKLVSWYTLMEGQ
EPIARKVVEQGMFVKHCKVEVYLTELKLCENGMNNAVTRRFSK
ADTIDTIEKEIRKIFSI PDEKETRLWNKYMSNTFEPLNKPDS TI
QDAGLYQGQVLVIEQKNEDGTWPRGPSTPNVKNSNYCLPSYTAY
KNYDYSEPGRNNEQPGLCGLSNLGNTCFMNSAIQCLSNTPPLTE
YFLNDKYQEELNFDNPLGMRGEIAKSYAELIKQMWSGKFSYVTP
RAFKTQVGRFAPQFSGYQQQDCQELLAFLLDGLHEDLNRIKKP
YIQLKDADGRPDKVVAEEAWENHLKRND SI IVDIFHGLFKSTLV
CPECAKISVTFDFPCYLTLPLPMKKERTLEVYLVRMDPLTKPMQ
YKVVPKIGNILDCTALSALSGIPADKMIVTDIYNHRFHRIFA
MDENLSSIMERDDIYVFEININRTEDTEHVIIPVCLREKFRHSS
YTHHTGSSLFGQPFLMAVPRNNTEDKLYNLLLLLRMCRYVKISTE
TEETEGSLHCKDQNINGNGPNGIHEEGSPSEMETDEPDDSSQ
DQELPSENENSQSEDSVGGDNDSENGLCTEDTCKGQLTGHKKRL
FTFQFNNGNTDINYIKDDTRHIRFDDRQLRLDERSFLALDWDP
DLKKRYFDENAAEDFEKHESVEYKPPKPFVKLKDCIELFTTKE
KLGAEDPWYCPNCKEHQQATKKLDLWVSLPVLVHLKRFYSRY
MRDKLDTLVDFPINDLDMSEFLINPNAGPCRYNLI AVSNHYGGM
GGGHYTAFAKNKDDGKWYFDDSSVSTASEDQIVSKAAVLFYQ
RQDTFSGTGFFPLDRETKGASAAATGIPLESDEDSNDNDNDIENE
NCMHTN

Native sequence Amino acids M1 – N952 (end residue) of human USP15.
Residue M243 of the fusion protein is equivalent to M1 of the
native enzyme. The GST tag is located at residues 1 - 220.

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Protease cleavage PreScission (LEVLFQGP) residues 221 - 228

Cloning sites *Not* 1 sites of pFastBac Dual.

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Nucleotide sequence of insert

gcggccgctATGGCGGAAGGCGGAGCGGCGGATCTGGACACCCAG
CGGTCTGACATCGCGACGCTGCTCAAAACCTCGCTCCGGAAAGGG
GACACCTGGTACCTAGTCGATAGTCGCTGGTTCAAACAGTGGAAA
AAATATGTTGGCTTTGACAGTTGGGACAAATACCAGATGGGAGAT
CAAAATGTGTATCCTGGACCCATTGATAACTCTGGACTTCTCAA
GATGGTGATGCCCAGTCACTTAAGGAACACCTTATTGATGAATTG
GATTACATACTGTTGCCAACTGAAGGTTGGAATAAACTTGTGAGC
TGGTACACATTGATGGAAGGTCAAGAGCCAATAGCACGAAAGGTG
GTTGAACAGGGTATGTTTGTAAAGCACTGCAAAGTAGAAGTATAT
CTCACAGAATTGAAGCTATGTGAAAATGGAAACATGAATAATGTT
GTAACCTCGAAGATTTAGCAAAGCTGACACAATAGATAACAATTGAA
AAGGAAATAAGAAAAATCTTCAGTATTCCAGATGAAAAGGAGACC
AGATTGTGGAACAAATACATGAGTAACACATTTGAACCACTGAAT
AAACCAGACAGCACCATTGAGGATGCTGGTTTATAACCAAGGACAG
GTATTAGTGATAGAACAGAAAAATGAAGATGGAACATGGCCAAGG
GGTCTTCTACTCCTAATGTGAAAAACTCAAATTACTGTCTTCCA
TCATATAACCGCTTATAAGAACTATGATTATTCGGAACCTGGAAGA
ACAATGAACAGCCAGGCCTCTGTGGCCTAAGTAACCTGGGAAAT
ACGTGTTTCATGAACTCAGCTATTCAGTGTTTGAGCAACACACCT
CCACTTACTGAGTATTTCCCTCAATGATAAGTATCAAGAAGAAGT
AATTTTGACAATCCCTTAGGAATGAGAGGTGAAATAGCTAAATCT
TATGCCGAAGTATCAAGCAAATGTGGTCTGGAAAGTTTAGCTAC
GTCACCCCAAGAGCCTTTAAGACACAGGTAGGACGTTTTGCACCT
CAGTTCCTCTGGATATCAGCAGCAAGACTGTCAAGAAGTGTAGCT
TTCCCTATTAGATGGATTACATGAGGATTTGAATAGAATTAGGAAA
AAACCATATATACAATTAAGATGCAGATGGAAGGCCAGATAAG
GTGGTTGCCGAAGAAGCCTGGGAAAACCATTTAAAACGAAATGAT
TCTATCATAGTAGATATATTTTCATGGCCTTTTCAAATCAACTTTA
GTTTGTCTGAGTGTGCTAAGATTTTCAGTAACATTTGATCCTTTT
TGTTACTTGACACTTCCATTGCCCATGAAAAAAGAACGCACCTTG
GAAGTTTACTTAGTTAGAATGGATCCGCTTACCAAACCTATGCAG
TACAAAGTGGTTGTCCCCAAAATTTGAAACATATTAGATCTTTGT
ACAGCATTGTCTGCTTTGTCAGGAATACCTGCAGATAAGATGATA
GTTACTGATATATACAATCATAGATTTTCACAGAATATTGCTATG
GATGAAAACCTTAGTAGTATTATGGAACGGGATGATATTTATGTG
TTTGAAATTAACATCAATAGGACAGAAGATACAGAGCACGTGATT
ATTCCTGTTTGCTAAGAGAAAAATTCAGACACTCGAGTTATAACC
CACCATACTGGTTCTTCACTTTTTGGTCAGCCCTTTCTTATGGCT
GTACCACGAAACAATACTGAAGACAACTTTATAATCTCCTGCTC
TTGAGAATGTGCCGATATGTCAAAATATCTACTGAACTGAAGAA
ACTGAAGGATCCCTACACTGCTGTAAGGACAAAATATTAATGGG
AATGGCCCAAATGGCATAACATGAAGAAGGCTCACCAAGTGAATG
GAAACAGATGAGCCAGATGATGAATCCAGCCAGGATCAAGAAGT
CCCTCAGAGAATGAAAACAGTCACTCTGAAGATTCAGTTGGAGGA
GATAATGATTCTGAAAATGGATTATGTACTGAGGATACTTGCAAA
GGTCAACTCACGGGACACAAAAACGATTGTTTACATTCAGTTC
AACAACCTTAGGCAATACTGATATCAACTACATCAAAGATGATACC
AGGCATATAAGATTTGATGATAGGCAGCTTAGGCTAGATGAAAGA
TCTTTTCTTGCTTTGGATTGGGATCCTGATTTGAAAAAAGATAT
TTTGATGAAAATGCTGCCGAGGACTTTGAAAAACATGAAAGTGTG
GAGTATAAACCTCCTAAAAAACCTTTGTGAAATTAAGATTGC
ATTGAACTTTTTACAACAAAAGAAAAGCTAGGTGCTGAAGATCCC
TGGTATTGTCCGAATTGTAAAGAACATCAGCAAGCCACAAAGAAA

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TTGGATTTATGGTCCCTGCCTCCAGTACTTGTAGTACATCTCAAG
CGATTTTCTTACAGTCGATACATGAGAGACAAGTTGGATACCTTA
GTTGATTTTCCTATCAATGACTTGGATATGTCGGAATTCTTAATT
AATCCAAATGCAGGTCCTTGCCGCTATAATCTGATTGCTGTTTCC
AACCCTATGGAGGGATGGGAGGAGGACACTATACTGCTTTTGCA
AAAAATAAAGATGATGGAAAATGGTACTATTTTGATGACAGTAGT
GTCTCCACTGCATCTGAAGACCAAATTGTGTCCAAAGCAGCATAT
GTACTCTTCTACCAGAGACAAGACACTTTCAGTGGAAGTGGCTTT
TTTCCTCTTGACCGAGAACTAAAGGTGCTTCAGCTGCCACTGGC
ATCCATTAGAAAGTGATGAAGATAGCAATGATAATGACAATGAT
ATAGAAAATGAAAAGTGTATGCACACTAACTaagcggccgc