

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active USP52 [486 - 924]

<u>Enzyme description:-</u>	USP52 [486 - 924]
<u>Clone number:-</u>	DU 75132
<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 76, 904.42 daltons
Average Mass 76, 954.65 daltons
[cysteines reduced, methionines have not been oxidised]

<u>Theoretical pI:-</u>	6.13
<u>Purity:-</u>	>80 %
<u>Activation protocol:-</u>	Constitutively active
<u>Enzyme storage buffer:-</u>	50 mM HEPES pH 7.5, 10% glycerol, 150mM NaCl, 1mM DTT
<u>Storage temperature:-</u>	-70 °C

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Clone Data Sheet

USP52 [486 - 924]

Protein USP52 [486 - 924]

Clone number DU 75132

Species Human

Accession number Q504Q3-1

Tags N-terminal GST

**Baculovirus
expressed protein**

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNK
KFELGLEFPNLPYYIDGDVKLTSMAIIRYIADKHNMLGGCPKE
RAEISMLEGAVLDIRYGVSR IAYSKDFETLKVDFLSKLPEMLKM
FEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKL
VCFKKRIEAIPOIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSD
LEVL FQGPLGSMVSKKYRKVTIKYSKL GLEDFDFKHYNKTLFAG
LEPHIPNAYCNCMIQVLYFLEPVRC LIQNHLCQKEFCLACELGF
LFHMLDLSRGDPCQGNNFLRAFRTIPEASALGLILADSDEASGK
GNLARLIQRWNRFLTQLHQDMQELEIPQAYRGAGGSSFCSSGD
SVIGQLFSCEMENCSLCRCGSETVRASSTLLFTLSYPDGSKSDK
TGKNYDFAQVLKRSICLDQNTQAWCDTCEKYOPTIQTRNIRHLP
DILVINCEVNSSKEADFWRMQAEVAFKMAVKKHGGEISKNKEFA
LADWKELGSPEGVLCPSIEELKNVWLPFSIRMKMTKNKGLDVC
NWTDGDEMQWGPAREEEHGVYVYDLMATVVHILDSRTGGSLVA
HIKVGETYHQKEGVTHQQWYLFNDFLIEPIDKHEAVQFDMNWK
VPAILYYVKR

Native sequence Amino acids M486 – R924 (end residue is L1202) of human USP52. Residue M232 of the fusion protein is equivalent to M486 of the native enzyme. The GST tag is located at residues 1 - 220.

Protease cleavage PreScission (LEVL FQGP) residues 221 - 228

Cloning sites *Bam*HI and *Not*I sites of pFastBac Dual.

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Nucleotide sequence

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAA
CCCCTCGACTTCTTTTGGAAATATCTTGAAGAAAAATATGAAGAG
CATTTGTATGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAG
TTTGAATTGGGTTTGGAGTTTCCCAATCTTCTTATTATATTGAT
GGTGATGTTAAATTAACACAGTCTATGGCCATCATACTTATATA
GCTGACAAGCACACATGTTGGGTGGTTGTCCAAAAGAGCGTGCA
GAGATTTCAATGCTTGAAGGAGCGGTTTGGATATTAGATACGGT
GTTTCGAGAATTGCATATAGTAAAGACTTTGAACTCTCAAAGTT
GATTTTCTTAGCAAGCTACCTGAAATGCTGAAAATGTTCTGAAGAT
CGTTTATGTCATAAAACATATTTAAATGGTGATCATGTAACCCAT
CCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTATACATG
GACCAATGTGCCTGGATGCGTTCCCAAATTAGTTTGTTTTAAA
AAACGTATTGAAGCTATCCACAAATTGATAAGTACTTGAAATCC
AGCAAGTATATAGCATGGCCTTTGCAGGGCTGGCAAGCCACGTTT
GGTGGTGGCGACCATCTCCAAAATCGGATCTGGAAGTTCTGTTT
CAGGGGCCCTGGGATCCATGGTTTCTAAGAAATACCGCAAGGTG
ACCATCAAATATTCCAAGCTAGGGCTGGAGGACTTTGACTTCAA
CACTACAATAAGACCTTGTGCTGGATTAGAGCCCCACATTCCC
AACGCCTACTGTAAGTGCATGATCCAGGTGCTCTATTTCTGGAG
CCTGTACGCTGTCTAATTCAAAACCACCTTTGCCAGAAGGAGTTC
TGTCTGGCATGTGAGCTGGGCTTCTGTTTACATGTTGGACCTC
TCTCGTGGTGACCCTTGCCAGGGCAATAATTTCTTCGGGCATTC
CGTACTATTCCTGAGGCCTCAGCCCTCGGTCTAATCCTGGCTGAC
TCAGATGAGGCCTCAGGCAAGGGCAATCTGGCCAGGCTCATTCAG
AGGTGGAATCGCTTCATTCTCACTCAACTGCATCAAGATATGCAG
GAGCTGGAAATACCACAGGCTTATCGAGGTGCTGGAGGCAGCAGC
TTTTGCTCATCGGGGACTCTGTTATTGGGCAGCTCTTCAGCTGT
GAGATGGAGAACTGCAGCCTCTGCCGCTGTGGCAGTGAGACCGTG
CGAGCCTCATCCACTCTGCTTTTACACTCTCCTACCCTGATGGT
AGCAAAGTGATAAACTGGGAAGAACTATGACTTTGCTCAGGTG
CTGAAGCGAAGCATCTGCCTGGACCAGAATACACAGGCCTGGTGT
GACACCTGTGAAAAGTACCAGCCCACGATTCAGACCCGCAACATC
CGCCATCTGCCAGATATTCTTGTCATCAATTGTGAGGTGAACAGC
TCAAAGAGGGCTGATTTCTGGAGAATGCAGGCTGAGGTTGCCTTC
AAGATGGCAGTAAAGAAACACGGTGGGGAAATCTCCAAGAACAAG
GAATTTGCTTTGGCTGATTGGAAGGAAGTGGGAGTCCAGAGGGT
GTGCTGGTGTGTCCCTCCATTGAGGAGTTGAAGAACGTCTGGCTT
CCTTTCTCCATTCGCATGAAGATGACCAAAAACAAGGGCTGGAT
GTTTGCAATTGGACTGATGGGGATGAGATGCAGTGGGGCCCAGCC
AGGGCAGAGGAGGAGCATGGTGTCTATGTGTATGACCTGATGGCT
ACTGTGGTACACATCCTGGACTCACGCACAGGGGGCAGCCTGGTG
GCTCACATCAAAGTTGGAGAGACCTACCACCAGCGCAAGGAGGGC
GTTACTCACCAGCAGTGGTATCTGTTCAATGACTTTCTTATTGAA
CCTATTGATAAGCATGAAGCTGTGCAGTTTGACATGAATTGGAAA
GTACCTGCAATCCTTTATTATGTCAAACGGTga