

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active USP48 [1 – 1035]

<u>Enzyme description:-</u>	USP48 [1 - 1035]
<u>Clone number:-</u>	DU 27626
<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 145, 762.79 daltons
Average Mass 145, 856.44 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.75

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,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

USP48 [1 - 1035]

Protein USP4 [1 - 1035]

Clone number DU 27626

Species Human

Accession number NM_0032236.7

Tags N-terminal GST

**Bacterially
expressed protein**

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA
VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFOGPLGSMAPRLQLEKAAWRW
AETVRPEEVSQEH IETAYRIWLEPCIRGVCRRNCKGNPNCLVGIGEH I W
LGEIDENS FHNIDDPNCERRKKN SFVGLTNLGATCYVNTFLQVWFLNLE
LRQALYLC PSTCS DYMLGDGIQEEKDYEPQ TIC EHLQYLFALLQNSNRR
YIDPSGFVKALGLDTGOQDAQEF SKLFMSLLED T LSKQKNPDVRNIVQ
QOFCGEYAYVTVCNQCGRESKLLSKFY E LELNIQGHKQLTDCISEFLKE
EKLEGDNRYFCENCQSKQ NATRKIRLLSLPCTLN LQLMRFVFDROTGHK
KKLNTYIGFSEILDMEPYVEHKGGSYVYELSAVLIHRGVSAYS GHYIAH
VKDPOSGEWYKFNDEDIEKMEGKKLQLGIEEDLAEPSKSQTRKPKCGK
THCSR NAYMLVYRLQTQEKPN TTVQVPAFLQELVDRDNSKFE EWCIEMA
EMRKQSV DKGKAKHEEVKELYQRLPAGAEPYEFVSLEWLQKWLDESTPT
KPIDNHACLCSHDKLHPDKISIMKRISEYAADIFYSRYGGPRLTVKAL
CKECVVERCRILRLKNQLNEDYKTVNNLLKAAVKGSDGFVVGKSSLRSW
RQLALEQLDEQDGAEQSNGKMNGSTLNKDESKEERKEEEEELNFNEDIL
CPHGELCISENERRLVSKEAWSKLQOYFPK AEPFPSYKECCSQCKILER
EGEENEALHKMIANEQK TSLPNL FQDKNRPCLSNWPEDTDVLYIVSQFF
VEEWRKFVRKPTRCSPVSSVGN SALLCPHGGLMFTFASMTKEDSKLIAL
IWPSEWQMIQKLFVVDHVIKITRIE VGDVNPSETQYI SEPKLCPECREG
LLCQQORDLREYTQATIYVHKVVDNKKVMKDSAPELNVSSSETEEDKEE
AKPDGEKDPDFNQSNGGTKRQKISHONYIAYQKQVIRRSMRHRKVRGEK
ALLV SANQTLKELKIQIMHAFSVAPFDQNL SIDGKILSDDCATLGT LGV
IPESVILLKADEPIADYAAMD DVMQVCMPEEGFKGTGLLGH

Native sequence Amino acids M1 – H1035 (end) of human USP48.
Residue M232 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 - 229

Cloning sites *Bam*H1 and *Not*1 sites of pFastBac Dual

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Nucleotide Sequence of insert

ggatccATGGCCCCGCGGCTGCAGCTGGAGAAGGCGGCCTGGCGCTGGGCGGAGACGGT
GCGGCCCCGAGGAGGTGTCGCAGGAGCACATCGAGACCGCTTACCGCATCTGGCTGGAGC
CCTGCATTCGCGGCGTGTGCAGACGAAACTGCAAAGGAAATCCGAATTGCTTGGTTGGT
ATTGGTGAGCATATTTGGTTAGGAGAAATAGATGAAAATAGTTTTTCATAACATCGATGA
TCCCAACTGTGAGAGGAGAAAAAGAACTCATTTGTGGGCCTGACTAACCTTGGAGCCA
CTTGTATGTCAACACATTTCTTCAAGTGTGGTTTTCTCAACTTGGAGCTTCGGCAGGCA
CTCTACTTATGTCCAAGCACTTGTAGTGACTACATGCTGGGAGACGGCATCCAAGAAGA
AAAAGATTATGAGCCTCAAACAATTTGTGAGCATCTCCAGTACTTGTTCCTTGTTC
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ACTGGACAACAGCAGGATGCTCAAGAATTTTCAAAGCTCTTTATGTCTCTATTGGAAGA
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GAGAATATGCCTATGTAAGTGTTCGCAACCAGTGTGGCAGAGAGTCTAAGCTTTTGTCA
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GGAATTTTTGAAGGAAGAAAAATTAGAAGGAGACAATCGCTATTTTTGCGAGAAGTGT
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CCTACGTGTATGAACTCAGCGCAGTCCCTCATAACAGAGGAGTGAAGTGTATTTCTGGC
CACTACATCGCCACGTGAAAGATCCACAGTCTGGTGAATGGTATAAGTTAATGATGA
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TGTGAAAGCCCTGTGTAAGGAATGTGTAGTAGAACGTTGTTCGCATATTGCGTCTGAAGA
ACCAACTAAATGAAGATTATAAACTGTTAATAATCTGCTGAAAGCAGCAGTAAAGGGC
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CCTTAAATAAAGATGAATCAAAGGAAGAAAGAAAAGAAGAGGAGGAATTAATTTTAAT
GAAGATATTTCTGTGTCCACATGGTGTGAGTTATGCATATCTGAAAATGAAAGAGGCTGT
TTCTAAAGAGGCTTGGAGCAAACCTGCAGCAGTACTTTCCAAAGGCTCCTGAGTTTCCAA
GTTACAAAGAGTGTGTTACAGTGCAAGATTTTGTAGAAAGAGAAGGGGAAGAAAATGAA
GCCTTACATAAGATGATTGCAAACGAGCAAAAGACTTCTCTCCCAAATTTGTTCCAGGA
TAAAAACAGACCGTGTCTCAGTAACTGGCCAGAGGATACGGATGTCTCTACATCGTGT
CTCAGTTCTTTGTAGAAGAGTGGCGGAAATTTGTTAGAAAGCCTACAAGATGCAGCCCT
GTGTCATCAGTTGGGAACAGTGTCTTTTTGTGTCCCCACGGGGCCCTCATGTTTACATT
TGCTTCCATGACCAAAGAAGATTCTAAACTTATAGCTCTCATATGGCCAGTGAGTGGC
AAATGATACAAAAGCTCTTTGTTGTGGATCATGTAATTAATAATCACGAGAATTGAAGTG
GGAGATGTAACCCTTCAGAAACACAGTATATTTCTGAGCCCAAACCTCTGTCCAGAATG
CAGAGAAGGCTTATTGTGTGTCAGCAGCAGAGGGACCTGCGTGAATACACTCAAGCCACCA
TCTATGTCCATAAAGTTGTGGATAATAAAAAGGTGATGAAGGATTCCGGCTCCGGAACTG
AATGTGAGTAGTTCTGAAACAGAGGAGGACAAGGAAGAAGCTAAACAGATGGAGAAAA
AGATCCAGATTTTAATCAAAGCAATGGTGGAAACAAAGCGCAAAAAGATATCCCATCAA
ATTATATAGCCTATCAAAGCAAGTTATTCGCCGAAGTATGCGACATAGAAAAGTTCGT
GGTGAGAAAGCACTTCTCGTTTTCTGCTAATCAGACGTTAAAAGAATTGAAAATTCAGAT
CATGCATGCATTTTTCAGTTGCTCCTTTTTGACCAGAATTTGTCAATTGATGGAAAGATTT
TAAGTGATGACTGTGCCACCCTAGGCACCCTTGGCGTCATTCTGAATCTGTCAATTTTA
TTGAAGGCTGATGAACCAATTGCAGATTATGCTGCAATGGATGATGTCATGCAAGTTTG
TATGCCAGAAGAAGGGTTTTAAAGGTACTGGTCTTCTTGGACATtaagcggccgc