

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active USP16 [1 – 823]

Enzyme description:- USP16 [1 - 823]

Clone number:- DU 46239

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 120, 317.52 daltons

Average Mass 120, 394.31 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.31

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

USP16 [1 - 823]

Protein USP16 [1 - 823]

Clone number DU 46239

Species Human

Accession number NM_006447.2

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAETSMLEGA
VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVL FQGPLGSMGKKRTKGKTVPID
DSSETLEPVCRHIRKGLEQGNLKKALVNVEWNICQDCKTDNKVKDKAAE
ETEEKPSVWLCLKCGHQGGRNSQE QHALKHYLTPRSEPHCLVLSLDNW
SVWCYVCDNEVQYCSSNQLGOVVDYVRKQASITTPKPAEKDNGNIELEN
KKLEKESKNEQEREKKNMAKENPPMNSPCQITVKGLSNLGNTCFFNAV
MQNLSQTPVLRRELLKEVKMSGTIVKIEPPDLALTEPLEINLEPPGPLTL
AMSQFLNEMQETKKGVVTPKELFSQVCKKAVRFKGYQQQDSQELLRYLL
DGMRAEEHQRVSKGILKAFGNSTEKLDEELKNKVVDYEKKSMPSFVDR
IFGGELTSMIMCDQCRTVSLVHESFLDLSLPVLDQSGKKS VNDKNLKK
TVEDEDQDSEEEKDNDSYIKERSDIPSGTSKHLQKKAKKQAKKQAKNQR
RQQKI QGKVLHLNDICTIDHPEDSEYEAEMSLQGEVNIKSNHISQEGVM
HKEYCVNQKDLNGQAKMIESVTDNQKSTEEVDMKNINMDNDLEVL TSSP
TRNLNGAYLTEGSNGEVDISNGFKNLNLNAALHPDEINIEILNDSHTPG
TKVYEVVNEDPETAFCTLANREVFNTDECSIQHCLYQFTRNEKLRDANK
LLCEVCTRRQCNGPKANIKGERKHVYTNAKKQMLISLAPPVLT LHLKRF
QQAGFNLRKVNKHIFPEILDLPFCTLKCKNVAEENTRVLYSLYGVVE
HSGTMRS GHYTAYAKARTANSHLSNLV LHGDIPODFEMESKGQWFHISD
THVQAVPTTKVLNSQAYLLFYERIL

Native sequence Amino acids M1 – L823 (end) of human USP16.
Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage PreScission (LEVL FQGP) residues 221 - 229

Cloning sites *Bam*H1 and *Not*1 sites of pGEX 6P-1

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Nucleotide Sequence of insert

ggatccATGGGAAAGAAACGGACAAAGGGAAAAACTGTTCCAATCGATG
ATTCTCTGAAACTTTAGAACCTGTGTGCAGACACATTAGAAAAGGATTG
GAACAAGGTAATTTGAAAAAGGCTTTAGTGAATGTGGAATGGAATATCTG
CCAAGACTGTAAGACTGACAATAAAGTGAAAGATAAAGCTGAAGAAGAAA
CAGAAGAAAAGCCTTCAGTTTGGCTGTGTCTTAAATGTGGCCATCAGGGC
TGTGGCAGAAATTCTCAGGAGCAGCATGCCTTGAAGCACTATCTGACGCC
AAGATCTGAACCTCACTGTCTGGTTCTTAGTTTGGACAACGGAGTGTAT
GGTCAAGTGGTTGATTATGTGAGGTCAGAAAACAAGCCAGCATTACAACCTCCAA
GCCAGCAGAGAAAAGATAATGAAAATATTGAACTTGAATAAAAAATTAG
AAAAAGAGAGTAAGAATGAACAAGAGAGAGAAAAAGGAAAAACATGGCT
AAAGAGAATCCTCCCATGAATTCTCCTTGCCAAATAACCGTGAAGGACT
CAGTAATTTGGGAAACACATGTTTCTTCAATGCAGTTATGCAGAACTTGT
CACAAACACCAGTGCTTAGAGAACTACTAAAAGAAGTGAAAATGTCTGGA
ACAATTTGTAATAAATTGAACCACCTGATTTGGCATTAAACAGAACCATTAGA
AATAAACCTTGAGCCTCCAGGCCCTCTTACTTTAGCCATGAGCCAGTTTC
TTAATGAGATGCAAGAGACCAAAAAGGGGGTTGTGACACCGAAAGAATC
TTTTCTCAGGTCTGTAAAAAGCAGTGCAGTTTAAAGGCTATCAGCAGCA
AGACAGCCAGGAGCTGCTTCGCTACTTATTGGATGGGATGAGAGCAGAAG
AACACCAAAGAGTGAGTAAAGGAATACTTAAAGCATTGGTAATTCTACT
GAAAAGTTGGATGAAGAACTAAAAAATAAAGTTAAAGATTATGAGAAGAA
AAAATCAATGCCAAGTTTTGTTGACCGCATCTTTGGTGGTAACTAAGTA
GTATGATCATGTGTGATCAATGCAGAAGTGTCTCCTTGGTTCATGAATCT
TTCCTTGATTTGTCCCTCCAGTTTTTAGATGATCAGAGTGGTAAGAAAAG
TGTAATGATAAAAAATCTGAAAAAGACAGTGGAGGATGAAGATCAAGATA
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ACAGAGGAAGTAGATATGAAAAATATCAACATGGATAATGATCTGGAGGT
TTTAACATCTTCTCCCACTAGGAATTTAAATGGTGCCTACCTAACGGAAAG
GGAGCAATGGAGAAGTGGACATTTCCAATGGTTTCAAAAACCTAAATTTG
AATGCTGCTCTTCATCCTGATGAAATAAATATAGAGATTCTGAATGATAG
TCATACTCCTGGAACAAAGGTGTATGAGGTTGTAAATGAAGATCCAGAAA
CTGCTTTCTGTACTCTTGCAACAGGGAAGTTTTCAATACTGATGAGTGT
TCAATCCAACATTGTTTATATCAGTTCACCCGTAATGAGAACTTCGAGA
TGCGAATAAACTGCTTTGTGAAGTATGCACACGGAGACAGTGAATGGAC
CAAAGGCAATATAAAAGGTGAAAGGAAGCATGTTTACACCAATGCCAAA
AAGCAGATGCTAATTTCTCTTGCTCCTCCTGTTCTTACTCTTCATTTAAA
GAGATTTACAGCAGGCTGGTTTTAACCTACGCAAAGTTAACAAACACATAA
AGTTTCCGAAATCTTAGATTTGGCTCCTTTTTGCACCCTTAAATGTAAG
AATGTTGCAGAAGAAAATACAAGGGTACTCTATTCTTATATGGAGTTGT
TGAACACAGTGGTACTATGAGGTCGGGGCATTACACTGCCATGCCAAGG
CAAGAACCACAAATAGTCATCTCTCTAATCTTTGTTCTTCACTGAGTAT
CCACAAGATTTTGAATGGAATCAAAAGGGCAGTGGTTTACATCAGCGA
CACACATGTGCAAGCTGTGCCTACAATAAAGTACTAAACTCACAAGCGT
ACCTCTATTTTATGAGAGAATACTGtaagcggccgc