

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active USP11 [1 – 963]

Enzyme description:- USP11 [1 - 963]

Clone number:- DU 20016

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 137, 532.41 daltons

Average Mass 137, 619.89 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.37

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

USP11 [1 - 963]

Protein USP11 [1 - 963]

Clone number DU 20016

Species Human

Accession number NM_001371072.1

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAETSMLEGA
VLDIRYGVSR IAYS KDFETLKVDLFLSKLPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFGQPLGSPGIPGSTRAAAMAV
APRLFGGLCFRFRDQNP E VAVEGR L P I S H S C V G C R R E R T A M A T V A A N P A
AAAAAVAAAAVTE D R E P Q H E E L P G L D S Q W R Q I E N G E S G R E R P L R A G E S
WFLVEKHWHYKQWEAYVQGGDQDSSTFPGCINNATLFODEINWRLKEGLV
EGEDYVLLPAAAWHYLVSWYGLEHGQPP I E R K V I E L P N I Q K V E V Y P V E L
LLVRHNDLGSHTVQFSHTDSIGLVLRTARERFLVEPQEDTRLWAKNSE
GSLDRLYDTHITVLDAALETGQLIIMETRKKDGTWPSAQLHVMNNMSE
EDEDFKGQPGICGLTNLGNTCFMNSALQCLSNVPQLTEYFLNNCYLEEL
NFRNPLGMKGEIAEAYADLVKQAWSGHRSIVPHVFNKNVGHFASQFLG
YQQHDSQELLSFLLDGLHEDLNRVKKKEYVELCDAAGRPDQEQEAWQ
NHKRRNDSVIVDTFHGLFKSTLVCPDCGNVSVTFDPFCYLSVPLPISHK
RVLEVFFIPMDPRRKPEQHRLVVPKKGKISDLCVALS KHTGISPERMMV
ADVFSHRFYKLYQLEEPLSSILDRDDIFVYEVSGRIEAIEGSREDIVVP
VYLRERTPARDYNNSYYGLMLFGHPLLVSVPDRDFTWEGLYNVLMYRLS
RYVTKPNSDDEDDGDEKEDDEEDKDDVPGPSTGGSLRDPEPEQAGPSSG
VTNRCPFLLDNCLGTSQWP P R R R R K Q L F T L Q T V N S N G T S D R T T S P E E V H
AQPYIAIDWEPKRYDEVEAEGYVKHDCVGYVMKKAPVRLQECIEL
FTTVETLEKENPWYCPSCQHQQLATKKLDLWMLPEILI IHLKRFSYTKF
SREKLDTLVEFPIRDLDFSEFVIQPNESNPELYKYDLIAVSNHYGGMR
DGHYTTFACNKDSGQWHYFDDNSVSPVNEQIESKAAYVLFYQRQDVAR
RLLSPAGSSGAPASPACSSPPSSEFMDVN

Native sequence Amino acids M1 – N963 (end) of human USP11.
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 - 229

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

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Nucleotide **Sequence of insert**

gcggccgctATGGCAGTAGCCCCGCGACTGTTTTGGGGGGCTCTGCTTCCGTTTCCGGGA
CCAGAATCCGGAAGTGGCTGTTGAGGGGCGTCTTCCAATCTCGCACAGCTGCGTTGGCT
GTAGAAGAGAACGGACGGCGATGGCGACGGTTCGCAGCAAATCCAGCTGCTGCTGCGGCG
GCTGTGGCGGCGGCAGCGCGGTGACTGAGGATAGAGAGCCACAGCACGAGGAGCTGCC
AGGCCTGGACAGCCAGTGGCGCCAGATAGAAAACGGCGAGAGTGGGCGAGAACGTCCAC
TGCGGGCCGGCGAAAGCTGGTTCCTTGTGGAGAAGCACTGGTATAAGCAGTGGGAGGCA
TACGTGCAGGGAGGGGACCAGGACTCCAGCACCTTCCCTGGCTGCATCAACAATGCCAC
ACTCTTTCAAGATGAGATAAACTGGCGCCTCAAGGAGGGACTGGTGAAGGCGAGGATT
ATGTGCTGCTCCCAGCAGCTGCTTGGCATTACCTGGTCAGCTGGTATGGTCTAGAGCAT
GGCCAGCCACCCATTGAACGCAAGGTCATAGAGCTGCCAACATCCAGAAGGTGCAAGT
GTACCCAGTAGAACTGCTGCTTGTCCGGCACAATGATTTGGGCAAATCTCACACTGTTT
AGTTCAGCCATACCGATTCTATTGGCCTAGTATTGCGCACAGCTCGGGAGCGGTTTTCTG
GTGGAGCCCCAGGAAGACACTCGGCTTTGGGCCAAGAACTCAGAAGGCTCTTTGGATAG
GTTGTATGACACACACATCACGGTTCTCGATGCGGCCCTTGAGACTGGGCAGTTGATCA
TCATGGAGACCCGCAAGAAAGATGGCACTTGGCCCAGCGCACAGCTGCATGTCATGAAC
AACAACATGTCGGAAGAGGATGAGGACTTCAAGGGTCAGCCAGGCATCTGTGGCCTCAC
CAATCTGGGCAACACGTGCTTCATGAACTCGGCCCTGCAGTGCCTCAGCAATGTGCCAC
AGCTCACCGAGTACTTCTCAACAACCTGCTACCTGGAGGAGCTCAACTTCCGCAACCCA
CTGGGCATGAAGGTGAGATCGCAGAGGCCATGCAGACCTGGTGAAGCAGGCGTGGTC
TGGCCACCACCGCTCCATTGTGCCACATGTGTTCAAGAACAAGGTTGGCCATTTTGCAT
CCCAATTTCTGGGCTACCAGCAGCATGACTCTCAGGAGCTGCTGTCAATTCCTCCTGGAC
GGGCTGCATGAGGACCTTAATCGGGTGAAGAAGAAGGAGTATGTGGAGCTGTGCGATGC
TGCTGGGCGACCGGATCAGGAGGTGGCACAGGAGGCATGGCAAACACAAACGGCGGA
ACGATTCTGTGATCGTGGACACTTTCCACGGCCTCTTCAAGTCCACGCTGGTGTGCCCC
GATTGTGGCAATGTATCTGTGACCTTCGACCCCTTCTGCTACCTCAGTGTTCCTACTGCC
TATCAGCCACAAGAGGGTCTTGGAGGTCTTCTTTATCCCATGGATCCGCGCCGCAAGC
CAGAGCAGCACCGGCTCGTGGTCCCCAAGAAAGGCAAGATCTCGGATCTATGTGTGGCT
CTGTCCAAACACACGGGCATCTCGCCAGAGAGGATGATGGTGGCTGATGTCTTCAGTCA
CCGCTTCTATAAGCTCTATCAGCTAGAGGAGCCTCTGAGCAGCATCTTGGACCGTGATG
ATATCTTCGTCTATGAGGTGTCAGGTCGCATTGAGGCCATTGAGGGCTCAAGAGAGGAC
ATCGTGGTTTCTGTCTACCTGCGGGAGCGCACCCCTGCCCGTGACTACAACAACCTCCTA
CTACGGCCTGATGCTTTTTTGGACACCCCTCCTGGTATCAGTGGCCCCGGGACCGCTTCA
CCTGGGAGGGCCTGTATAACGTCTGATGTACCGGCTCTCACGCTACGTGACCAAACCC
AACTCAGATGATGAGGACGATGGGGATGAGAAAGAAGATGACGAGGAGGATAAAGATGA
CGTCCCTGGGCCCTCAACTGGGGGCAGCCTCCGAGACCCTGAGCCAGAGCAGGCTGGGC
CCAGCTCTGGAGTCACGAACAGGTGCCCGTTCTCCTCGGACAATTGCCCTTGGCACATCT
CAGTGGCCCCAAGGCGACGACGCAAGCAGCTGTTACCCTGCAGACGGTGAACCTCAA
TGGGACCAGCGACCGCACAACTCCCCTGAAGAAGTCCATGCCAGCCGTACATTGCTA
TCGACTGGGAGCCAGAGATGAAGAAGCGTTACTATGACGAGGTAGAGGCTGAGGGCTAC
GTGAAGCATGACTGCGTCGGGTACGTGATGAAGAAGGCTCCCCTGCGGCTGCAGGAGTG
CATTGAGCTCTTACCCTGTGGAGACCCTGGAGAAGGAAAACCCCTGGTACTGCCCTT
CCTGCAAGCAGCACCAGCTGGCAACCAAGAAGCTGGACCTGTGGATGCTGCCGGAGATT
CTCATCATCCACCTGAAACGCTTTTTCTACACCAAGTTCTCCGAGAGAAGCTGGACAC
CCTCGTGGAGTTTCTATCCGGGACCTGGACTTCTCTGAGTTTGTTCATCCAGCCACAGA
ATGAGTCGAATCCGGAGCTGTACAAATATGACCTCATCGCGGTTTCCAACATTATGGG
GGCATGCGTGATGGACACTACACAACATTTGCCTGCAACAAGGACAGCGGCCAGTGGCA
CTACTTTGATGACAACAGCGTCTCCCCTGTCAATGAGAATCAGATCGAGTCCAAGGCAG
CCTATGTCTCTTCTACCAACGCCAGGACGTGGCGCGACGCTGCTGTCCCCGGCCGGC
TCATCTGGCGCCCCAGCCTCCCCTGCCTGCAGCTCCCCACCCAGCTCTGAGTTCATGGA
TGTTAATt gaggccg