

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of active USP26 [1 – 835 mouse]**

**Enzyme description:-** USP26 [1 - 835]

**Clone number:-** DU 63470

**Source:-** Recombinant

**Expression system:-** *E.coli*

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Calculated molecular mass:-**

Monoisotopic 122, 778.11 daltons

Average Mass 122, 856.16 daltons

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 6.83

**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**

**USP26 [1 - 835]**

**Protein** USP26 [1 - 835]

**Clone number** DU 63470

**Species** Mouse

**Accession number** AF285570.1

**Tags** N-terminal GST

**Bacterially expressed protein**

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG  
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAETSMLEGA  
VLDIRYGVSR IAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH  
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS  
KYIAWPLQGWQATFGGGDHPPKSDLEVL FQGPLGSPEFATMEPILINAQ  
**VQMWSAKAGMSKSRNALIETCVGKREVKLILYFSTGKIKTLQLHDNIKS**  
**VVLQTYGEDQNYLHLTFKNNDLFLVEKLTITTDARRLKRFLDKTSQGSIR**  
**PARSDERC GEPSTSAQELNGSGSSCETNSECFESPKESEMCMFREL SLL**  
**PSSSTFLHNVGLENQFIKRKRFFSDLAKNEKQSNLKD SIRDFEANLVV**  
**CISNEKGKERNVREVDISKPGFGFPFETNYPEDSGVDVRLNDLITKLF**  
**SPVLETHCIENGLEWHEYMKTYLLYPEKLWQGLPNVGNTCYINVVLQS**  
**LCSIPLFINDLFNQGF PWIKAPKDDFNMLLMQLLV LKDIYNARFRQKLL**  
**IGITKALPIFGEIFAVDRQND AHEFLSLCLVQLKETFORVTMMWQSEND**  
**SGDFYLLKDI FADYATINKMPVCPVTNNFEFELLSSIFCKACGLTLFKG**  
**EPSRYLSINIPQGGKDMSIQSTLDLFFSAEELEHRCEKCLYNKSVSFHR**  
**FGRLPRVIIVHLKRYHFNESWVMKKDERPILVSKYLRLSCHCSKSTKPP**  
**PPLRPGEHVKNLDLLKPLEVLGSEILKLPFNSVRTSRSKGFETINITSN**  
**RESEAQSGKRVSEVLSGKVQOENSGKGDTAHIVGSELTKETEKLKKHEE**  
**EHRPSDLDSGSIREAQKYQQA EKCN EGRSDKQISLEALTQSRPKPISQE**  
**QTENLGKTTLSHTQDSSQSSQSSSDSSKSSRCSDDL DKKAKPTRKVDPT**  
**KFNKKEDNVYRLVNIINHIGNSPNGGHYINDAFDFKRQSWFTYSDLHVT**  
**RTQEDFVYGRSSTGYVFFYMHNDIFEELLAKETQSTSTSKG**

**Native sequence** Amino acids M1 – G835 (end) of mouse USP26.  
Residue M237 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** *Eco*R1 and *Not*I sites of pGEX 6P-1

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### Nucleotide Sequence of insert

gaattcgccaccATGGAGCCCATATTGATAAATGCTCAAGTCCAGATGTGGAGTGCAAA  
GGCAGGAATGTCCAAGTCAAGAAATGCACTCATTGAAACATGTGTAGGAAAACGAGAAG  
TTAAACTTATTCTCTATTTTCAGCACTGGAAAGATTAAGACTTTGCAACTACACGATAAT  
ATTTAAAAGTGTGGTCCTTCAAACCTATGGCGAAGACCAGAATTACCTACATTTGACTTT  
TAAAAATAATGATTTCTTGTTTTGTTGAGAACTCACCACCACAGATGCCAGAAGACTGA  
AGAGATTTCTAGACAAAACCTCTCAAGGTAGTATTTCGGCCAGCCAGAAGTGATGAGAGA  
TGTGGTGAGCCTAGCACAAAGTGCACAGGAGTTGAATGGCTCTGGAAGTTCATGTGAAAC  
AAATAGTGAGTGCTTTGAATCACCCAAAGAAAGTGAAATGTGCATGTTTCGTGAGTTGT  
CTTTGCTTCCATCCTCATCAACCTTTCTTCACAATGTAGGATTATTAGAAAACCAATTC  
ATAAAGAGGAAAAGATTTTTCTCTGATTTAGCAAAAAATGAAAAACAGAGCAACCTGAA  
GGACAGTATCAGGGACTTTGAGGCAAATTTAGTGGTGTGTATCTCTAATGAAAAGGGAA  
AAGAAAGGAATGTAAGAGAAGTAGACATCAGTAAGCCAGGGTTTGGATTTCCATTTGAG  
ACCAACTATCCTGAAGATAGTGGTGTGGATGTTTCGTGATCTTAATGATCTCATTACAAA  
ATTATTTTCTCCAGTTCTGTTGGAAACACACTGTATTGAGAACGGCCTAGAGTGGCATG  
AATATATGAAGACATACTTGCTTTACCCAGAGAAATTTGTGGCAAGGCCTGCCTAATGTG  
GGAAACACCTGCTATATAAATGTTGTATTACAGTCTCTATGCTCAATACCACTGTTTTAT  
TAATGATTTATTCAACCAGGGTTTCCCATGGATTAAGCTCCCAAAGATGATTTTAACA  
TGCTCTTGATGCAACTGCTTGTTTTGAAAGATATTTACAACGCAAGATTTAGACAGAAG  
TTACTTATAGGTATTACAAAAGCCCTCCCATATTTGGAGAGATATTTGCTGTTGACAG  
GCAGAATGATGCTCATGAGTTTTTAAGTCTCTGTTTAGTTCAGTTGAAGGAGACTTTCC  
AAAGAGTAACCATGATGTGGCAGTCTGAAAATGATTCGGGGGATTTTTACTTACTTAAA  
GACATTTTTGCTGATTATGCTACTATCAACAAAATGCCCGTTTGTCTGTTACCAATAA  
TTTTGAATTTGAGTTGCTAAGCTCCATTTTTTTGTAAAGCTTGTGGCCTGACTCTTTTTTA  
AGGGAGAACCAAGTAGATACCTTTCTATCAACATTCCCAAGGAGGGAAAGACATGTCC  
ATCCAGTCCACTTTAGATCTTTTTCTTTAGTGCAGAGGAGCTTGAGCATAGGTGTGAAAA  
GTGTTTGTACAACAAATCTGTTTCATTTACAGGTTTGGCCGGCTACCCAGGGTAATTA  
TTGTTTCATCTGAAACGCTATCACTTTAATGAGTCATGGGTAATGAAGAAGGATGAGCGG  
CCCATCCTTGTTTCCAAATACTTAAGGCTGTCTTGTCACTGTAGCAAAAAGCACAAAACC  
GCCCCACCCCTTCGCCCAGGTGAACATGTTAAGAATCTTGACTTATTTAAAACCCCTTG  
AAGTGTGGGTTCGGAAATACTCAAATTCGCTTTTTAATTCAGTGAGGACCTCTAGATCC  
AAGGGTTTTCGAAACTATAAACATCACATCAAACAGGGAGTCAGAAGCACAAAGTGGGAA  
AAGAGTCTCTGAAGTGTGAGTGGAAAAGTGCAGCAGGAAAATTCAGGGAAAGGTGACA  
CAGCACATATAGTTGGGTGAGAACTTACAAAGGAGACTGAGAAACTCAAGAAACATGAG  
GAAGAGCATAGACCCAGTGATTTAGATTTCTGGTAGTATCAGGGAGGCCAAAAGTACCA  
ACAGGCTGAGAAATGTAACGAAGGGAGAAGTGATAAGCAGATTTCCCTAGAGGCACTTA  
CTCAAAGCCGTCCAAAACCAATCTCCAGGAACAGACAGAAAACCTTGGGAAAACCTACA  
CTGTCACATACCCAGGATAGTAGTCAGAGTTCACAGAGCTCATCAGATTCCAGTAAGAG  
CTCCCGATGCAGTGATGATCTCGATAAGAAGGCAAAGCCTACACGCAAGGTGGATCCAA  
CAAAGTTTTAATAAAAAAGAAGATAATGTTTACAGGCTTGTTAATATTATCAACCATATT  
GGGAACAGTCCCAATGGAGGCCACTACATCAATGATGCCTTTGACTTCAAGAGGCAGAG  
TTGGTTCACTTATAGTGATCTACATGTAACAAGAACCAAGAGGACTTTGTATATAGGG  
GTCGGAGTTCTACTGGGTATGTCTTCTTTTACATGCATAATGATATATTTGAAGAGCTC  
TTGGCAAAGGAAACTCAGTCTACCAGCACATCCAAGGGTtagcgccgc