

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of active USP20 [1 - 914]**

|                                     |                                      |
|-------------------------------------|--------------------------------------|
| <b><u>Enzyme description:-</u></b>  | USP20 [1 - 914]                      |
| <b><u>Clone number:-</u></b>        | DU 15664                             |
| <b><u>Source:-</u></b>              | Recombinant                          |
| <b><u>Expression system:-</u></b>   | Baculovirus expression vector system |
| <b><u>Tag:-</u></b>                 | N-terminal GST                       |
| <b><u>Purification method:-</u></b> | GSH Sepharose                        |

#### **Calculated molecular mass:-**

Monoisotopic      128, 629.86 daltons  
Average Mass      128, 712.17 daltons  
[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-**                      5.78

**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,  
10 mM DTT

**Storage temperature:-**                      -70 °C

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**Clone Data Sheet**

**USP20 [1 - 914]**

**Protein** USP20 [1 - 914]

**Clone number** DU 15664

**Species** Human

**Accession number** Q9Y2K6

**Tags** N-terminal GST

**Baculovirus  
expressed protein**

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNK  
KFELGLEFPNLPYYIDGDVKLTSMAIIRYIADKHNMLGGCPKE  
RAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKM  
FEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKL  
VCFKKRIEAIPOIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSD  
**LEVLFGQPLGSMGDSRDLCPHLDSIGEVTKEDLLLKSKGTCQSC**  
**GVTGPNLWACLQVACPYVGCGESFADHSTIHAQAKKHNLTVNLT**  
**TFRLWCYACEKEVFLEQRLAAPLLGSSSKFSEQDSPPSHPLKA**  
**VPIA VADEGESESEDDDLKPRGLTGMKNLGNSCYMNAALQALS**  
**CPPLTQFFLECGGLVRTDKKPALCKSYQKLVSEVWHKKRPSYVV**  
**PTSLSHGIKLVNPMFRGYAQODTQEFRLCLMDQLHEELKEPVVA**  
**TVALTEARDSDSSDTDEKREGDRSPSEDEFLSCDSSSDRGE**  
**QGRGGSSQAETELLIPDEAGRAISEKERMKDRKFSWGQORTNS**  
**EQVDEADVDTAMAALDQPAEAQPPSPRSSPCRTPEPDND AHL**  
**RSSSRPCSPVHHHEGHAKLSSSPRASPV RMAPSYVLKKAQVLS**  
**AGSRRRKEQRYRSVISDIFDGSILSLVQCLTCDRVSTTVETFQD**  
**LSLPIPGKEDLAKLHSAIYQNVPAKPGACGDSYAAQGWLAFIVE**  
**YIRRFVVSCTPSWFWGPPVVTLEDCLAFFAADELKGDNMYSCER**  
**CKKLRNGVKYCKVLRRLPEILCIHLKRFRHEVMYSFKINSHVSFP**  
**LEGLDLRPFLAKECTSQITTYDLSVICHHGTAGSGHYIAYCQN**  
**VINGQWYEFDDQYVTEVHETVVQNAEGYVLFYRKSSEEAMRERQ**  
**QVVSLAAMREPSLLRFYVSREWL NKFNTFAEPGPITNQTF LCSH**  
**GGIPPHKYHYIDDLVVILPQNVWEHLYNRFGGGPAVNHLVCSI**  
**CQVEIEALAKRRRIEIDTFIKLNKAFQAEESPGVIYICISMQWFR**  
**EWEAFVKGKDNEPPGPIDNSRIAQVKGSGHVQLKQGADYQOISE**  
**ETWTYLNLSLYGGGPEIAIRQSV AQPLGPENLHGEQKIEAETRAV**

**Native sequence** Amino acids M1 – V914 (end residue) of human USP20.  
Residue M234 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 - 228

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

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### **Nucleotide sequence of insert**

ggatccATGGGGGACTCCAGGGACCTTTGCCCTCACCTTGACTCCATAG  
GAGAGGTGACCAAAGAGGACTTGCTGCTCAAATCTAAGGGAACCTGTCA  
GTCGTGTGGGGTCACCGGACCAAACCTATGGGCCTGTCTGCAGGTTGCC  
TGCCCCATGTTGGCTGCGGAGAATCCTTCGCTGACCACAGCACCATT  
ATGCACAGGCAAAAAGCACAACTTGACCGTGAACCTGACCACGTTCCG  
ACTGTGGTGTACGCCCTGTGAGAAGGAGGTATTCTTGAGCAGCGGCTG  
GCAGCCCCCTCTGCTGGGCTCCTCTTCCAAGTTCTCTGAACAGGACTCCC  
CGCCACCCTCCACCCCTCTGAAAGCTGTTCCCTATTGCTGTGGCTGATGA  
AGGAGAGTCTGAGTCAGAGGATGATGACCTGAAACCTCGAGGCCTCACG  
GGCATGAAGAACCTCGGGAACCTCTGCTACATGAACGCCGCCCTGCAGG  
CCCTGTCCAATTGCCCGCCGCTGACTCAGTTCTTCTTGAGTGTGGCGG  
CCTGGTGCACAGATAAGAAGCCAGCCCTGTGCAAGAGCTACCAGAAG  
CTGGTCTCTGAGGTCTGGCATAAGAAACGGCCAAGCTACGTGGTCCCCA  
CCAGTCTGTCTCATGGGATCAAGTTGGTCAACCCAATGTTCCGAGGCTA  
TGCCAGCAGGACACCCAAGAGTTCTTCGCTGCCTGATGGACCAGCTG  
CACGAGGAGCTCAAGGAGCCGGTGGTGGCCACGGTGGCGCTGACGGAGG  
CTCGGGACTCAGATTCGAGTGACACGGATGAGAAACGGGAGGGTGACCG  
GAGCCCATCAGAAGATGAGTTCTTGTCTGTGACTCGAGCAGTGACCGG  
GGTGAGGGTGCAGGGCAGGGCGTGGCGGGGGCAGCTCGCAGGCCGAGA  
CGGAGCTGCTGATCCCAGATGAGGCGGGCCGAGCCATCTCTGAGAAGGA  
GCGGATGAAGGACCGCAAGTTCTCCTGGGGCCAGCAGCGTACAAACTCG  
GAGCAAGTGGACGAGGACGCTGATGTGGACACTGCCATGGCTGCCCTTG  
ACCAGCCCGGGAGGCCAGCCCCGTCACCACGGTCTCCAGCCCCCTG  
CCGACGCCAGAGCCGACAATGATGCTCACCTACGCAGCTCCTCTCGC  
CCCTGCAGCCCCGTCCACCACCAGAGGGCCATGCCAAGCTGTCTAGCA  
GCCCCCTCGTGCAAGCCCCGTGAGGATGGCACCGTCTGCTGCTCAA  
GAAAGCCCAGGTATTGAGTGTGGCAGCCGGAGGCGGAAGGAGCAGCGC  
TACCGCAGCGTCATCTCAGACATCTTTGACGGTCCATTCTCAGCCTCG  
TGCAGTGTCTCACCTGTGACCGGGTATCCACCACAGTGGAAACGTTCCA  
GGACTTATCACTGCCCATTCCTGGAAAGGAGGACCTGGCCAAGCTCCAT  
TCAGCCATCTACCAGAATGTGCCGGCCAAGCCAGGCGCCTGTGGGGACA  
GCTATGCCGCCAGGGCTGGCTGGCCTTCATTGTGGAGTACATCCGACG  
GTTTGTGGTATCCTGTACCCCCAGCTGGTTTTTGGGGGCCTGTCTGACC  
CTGGAAGACTGCCTTGCTGCCTTCTTTGCCGCTGATGAGTTAAAGGGTG  
ACAACATGTACAGCTGTGAGCGGTGTAAGAAGCTGCGGAACGGAGTGAA  
GTACTGCAAAGTCTGCGGTTGCCCGAGATCCTGTGCATTACCTAAAG  
CGTTTTCGGCACGAGGTGATGTACTCATTCAAGATCAACAGCCACGTCT  
CCTTCCCCCTCGAGGGGCTCGACCTGCGCCCCCTTCTTGCCAAGGAGTG  
CACATCCCAGATCACCACCTACGACCTCCTCTCGGTCATCTGCCACCAC  
GGCACGGCAGGCAGTGGGCACTACATCGCCTACTGCCAGAAGCTGATCA  
ATGGGCAGTGGTACGAGTTTGATGACCAGTACGTACAGAAAGTCCACGA  
GACGGTGGTGCAGAACGCCGAGGGCTACGTACTCTTCTACAGAAAGAG  
AGCGAGGAGGCCATGCGGGAGCGACAGCAGGTGGTGTCCCTGGCCGCCA  
TGCGGGAGCCCAGCCTGCTGCGGTTCTACGTGTCCCAGGAGTGGCTCAA  
CAAGTTCAACACCTTCGCGGAGCCAGGCCCCATCACCAACCAGACCTTC  
CTCTGCTCCCACGGAGGCATCCCGCCCCACAAATACCACTACATCGACG  
ACCTGGTGGTCATCCTGCCCCAGAAGCTCTGGGAGCACCTGTACAACAG  
ATTCGGGGGTGGCCCCGCCGTGAACCACCTGTACGTGTGCTCCATCTGC  
CAGGTGGAGATCGAGGCACTGGCCAAGCGCAGGAGGATCGAGATCGACA  
CCTTCATCAAGTTGAACAAGGCCTTCCAGGCCGAGGAGTCCCGGGCGT  
CATCTACTGCATCAGCATGCAGTGGTTCGGGAGTGGGAGGCGTTTCGTC  
AAGGGGAAGGACAACGAGCCCCCGGGCCATTGACAACAGCAGGATTG  
CACAGGTCAAAGGAAGCGGCCATGTCCAGCTGAAGCAGGGAGCTGACTA  
CGGCAGATTTCCGAGGAGACCTGGACCTACCTGAACAGCCTGTATGGA  
GGTGGCCCCGAGATTGCCATCCGCCAGAGTGTGGCGCAGCCGCTGGGCC  
CAGAGAACCCTGCACGGGGAGCAGAAGATCGAAGCCGAGACGCGGGCCGT  
Gtgagcggccgc

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