

## *MRCPPU Reagents and Services*

### **Standard Operating Procedure**

#### **Preparation of USP1 [1 – 785]**

<b><u>Enzyme description:-</u></b>	USP1 [1 - 785] G679A G671A
<b><u>Clone number:-</u></b>	DU 23019
<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
<b><u>Calculated molecular mass:-</u></b>	
Monoisotopic	114, 841.75 daltons
Average Mass	114, 914.18 daltons
[cysteines reduced, methionines have not been oxidised]	
<b><u>Theoretical pI:-</u></b>	5.44
<b><u>Purity:-</u></b>	75 %
<b><u>Activation protocol:-</u></b>	Constitutively active
<b><u>Enzyme storage buffer:-</u></b>	
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	
<b><u>Storage temperature:-</u></b>	-70 °C

# *MRCPPU Reagents and Services*

## Clone Data Sheet

### USP1 [1 – 785]

<b><u>Protein</u></b>	USP1 [1 – 785] G670A G671A
<b><u>Clone number</u></b>	DU 23019
<b><u>Species</u></b>	Human
<b><u>Accession number</u></b>	NP_003359.3
<b><u>Tags</u></b>	N-terminal GST
<b><u>Bacterially expressed protein</u></b>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDENLYFQGGSMPGVIPSSENGLSRGS PSKKNRLSLKFFQKKETKRALDFTDSQENEEKASEYRASEIDQVVPAAQ SSPINCEKRENLLPFVGLNNLGNTCYLNSILQVLYFCPGFKSGVKHLEN IISRKKEALKDEANQKDKGNCKEDSLASYELICSLQSLIISVEQLQASF LLNPEKYTDELATQPRLLNLTRELNPMYEGYLQHDAQEVLQOCILGNIQ ETCQLLKKEEVKNVAELPTKVEEIPHPKEEMNGINSIEMDSMRHSEDFK EKLPGKNGKRKSDTEFGNMKKKVKLSKEHQSLLENQRQTRSKRKATSDT LESPPKII PKYI SENESPRPSQKKS RVKINWLKSATKQPSILSKFCSLG KITTNQGVKGQSKENECDPEEDLGKCESDNTTNGCGLES PGNTVTPVNV NEVKPINKGEEQIGFELVEKLFQGGQLVLRTRCLECESLTERREDFQDIS VPVQDELSKVEESSEISPEPKTEMKTLRWAI SQFASVERIVGEDKYFC ENCHHYTEAERSLLFDKMPEVIT IHLKCF AASGLEFDCYGGGLSKINTP LLTPLKLSLEEWSTKPTNDSYGLFAVVMHSGITISSGHYTASVKVTDLN SLELDKGNFVVDQMC EIGKPEPLNEEEARGVVENYNDEEVSIRVGGNTQ PSKVLNKKNVEAIGLLAAQSKADYELYNKASNPDKVASTAFAENRNSE TSDTTGT HESDRNKESSDQTGINISGFENKISYVVQSLKEYEGKWLLFD DSEVKVTEEKDFLNSLSPSTSPSTPYLLFYKKL</p>
<b><u>Native sequence</u></b>	<p>Amino acids M1 – L785 (end) of human USP1. Residue M230 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.</p>

The protein has a G670A and G671A substitution. Residue G670 and G671 are equivalent to A899 and A900 of the fusion protein.

## *MRCPPU Reagents and Services*

**Protease cleavage**      TEV Protease (ENLYFQG) residues 221 - 227

**Cloning sites**            *Bam*H1 and *Not*1 sites of pFastBac TEV2

## *MRCPPU Reagents and Services*

### **Nucleotide** **Sequence of insert**

ggatccATGCCTGGTGTCCATACCTAGTGAAAGTAATGGACTTTCAAGAGGTAGCCCTTC  
AAAGAAAAACAGACTTTTCCTTAAAGTTTTTTTCAGAAAAAGGAACTAAGAGAGCTTTGG  
ATTTACAGATTCTCAAGAAAATGAAGAAAAAGCTTCTGAATATAGAGCATCTGAAATT  
GATCAAGTTGTTCCCTGCAGCACAGTCTTCCCTATAAACTGTGAGAAGAGAGAAAACCTT  
GTTACCATTTGTGGGACTGAATAATCTCGGCAATACTTGCTATCTTAATAGTATACTTC  
AGGTATTATATTTTTGTCCCGTTTTTAAATCTGGAGTAAAGCACTTATTTAATATTATT  
TCAAGGAAGAAAGAAGCTCTAAAGGATGAAGCCAATCAAAAAGACAAGGGAAATTGCAA  
AGAAGATTCTTTGGCAAGTTATGAATTGATATGCAGTTTACAGTCCTTAATCATTTCGG  
TTGAACAGCTCCAGGCTAGTTTTCTCTTAAATCCAGAGAAATATACTGATGAACTTGCC  
ACTCAGCCAAGGCGACTGCTTAACACACTGAGGGAACCTCAACCCTATGTATGAAGGATA  
TCTACAGCATGATGCACAGGAAGTATTACAATGTATTTTGGGAAACATTCAAGAAACAT  
GCCAACTCCTAAAAAAGAAGAAGTAAAAAATGTGGCAGAATTACCTACTAAGGTAGAA  
GAAATACCTCATCCGAAAGAGGAAATGAATGGTATTAACAGCATAGAGATGGACAGTAT  
GAGGCATTCTGAAGACTTTAAAGAGAACTCCCAAAGGAAATGGGAAAAGAAAAAGTG  
ACACTGAATTTGGTAACATGAAGAAAAAAGTTAAATTATCCAAGGAACACCAGTCATTG  
GAAGAGAACCAGAGACAACTAGATCAAAAAGAAAAGCTACAAGTGATACATTAGAGAG  
TCCTCCTAAAATAATTCCAAGTATATTTCTGAAAATGAGAGTCCAAGACCCTCACAAA  
AGAAATCAAGAGTTAAAATAAATTGGTTAAAGTCTGCAACTAAGCAACCCAGCATTCTT  
TCTAAATTTTGTAGTCTGGGAAAAATAACAACAACCAAGGAGTCAAAGGACAATCTAA  
AGAAAATGAATGTGATCCTGAAGAGGACTTGGGGAAGTGTGAAAGTGATAACACAAC  
ATGGTTGTGGACTTGAATCTCCAGGAAATACTGTTACACCTGTAAATGTTAATGAAGTT  
AAACCATAAACAAAGGTGAAGAACAAATTGGTTTTGAGCTAGTGGAGAAATTATTTCA  
AGGTCAGCTGGTATTAAGGACGCGTTGCTTGGAAATGTGAAAGTTTAAACAGAAAGAAGAG  
AAGATTTTCAAGACATCAGTGTGCCAGTACAAGAAGATGAGCTTTCCAAAGTAGAGGAG  
AGTTCTGAAATTTCTCCAGAGCCAAAAACAGAAATGAAGACCCTGAGATGGGCAATTTT  
ACAATTTGCTTCAGTAGAAAGGATTGTAGGAGAAGATAAATATTTCTGTGAAAACCTGCC  
ATCATTATACTGAAGCTGAACGAAGTCTTTTGTGTTGACAAAATGCCTGAAGTTATAACT  
ATTCATTTGAAGTGCTTTGCTGCTAGTGGTTTTGGAGTTTGATTGTTATGGTGGTGGACT  
TTCCAAGATCAACACTCCTTTATTGACACCTCTTAAATTGTCACTAGAAGAATGGAGCA  
CAAAGCCAACCTAACGACAGCTATGGATTATTTGCGGTTGTGATGCATAGTGGCATTACA  
ATTAGTAGTGGGCATTACACTGCTTCTGTAAAGTCACTGACCTTAACAGTTTGAAGT  
AGATAAAGGAAATTTTGTGGTTGACCAAATGTGTGAAATAGGTAAGCCAGAACCATTGA  
ATGAGGAGGAAGCAAGGGGTGTGGTTGAGAATTATAATGATGAAGAAGTGTCAATTAGA  
GTTGGTGGAAATACACAGCCAAGTAAAGTTTTGAACAAAAAATGTAGAAGCTATTGG  
ACTTCTTGcAGcACAAAAGAGCAAAGCAGATTATGAGCTATACAACAAAGCCTCTAATC  
CTGATAAGGTTGCTAGTACAGCGTTTTGCTGAAAATAGAAATCTGAGACTAGTGATACT  
ACTGGGACCCATGAATCTGATAGAAACAAGGAATCCAGTGACCAAACAGGCATTAATAT  
TAGTGGATTTGAGAACAAAATTTTCATACGTAGTGCAAAGCTTAAAGGAGTATGAGGGGA  
AGTGGTTGCTTTTTGATGATTCTGAAGTCAAAGTTACTGAAGAGAAGGACTTTCTGAAT  
TCTCTTTCCCCTTCTACATCTCCTACTTCTACTCCTTACTTGCTATTTTATAAGAAATT  
Ataggcggccgc