MRC PPU REAGENTS

Standard Operating Procedure

Preparation of vOTU [1 - 183]

Enzyme description:- vOTU [1 – 183]

Clone number:- DU 45351

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 47, 629.93 daltons
Average Mass 47, 660.62 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.16

Purity:- >80 %

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, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 deg C

MRC PPU REAGENTS

Clone Data Sheet

vOTU [1 – 183]

Protein vOTU [1 – 183]

Clone number DU 45351

Species Crimean-Congo Hemorrhagic fever virus strain IbAr10200

Accession number 3ZNH_A [GI:453055625]

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MDFLRSLDW**
TQVIAGQYVSNPRFNISDYFEIVRQPGDGNCFYHSIAELTMPNKTDHS
YHYIKRLTESAARKYYQEEPEARLVGLSLEDYLKRMLSDNEWGSTLEA
SMLAKEMGITIIIWTVAASDEVEAGIKFGDGDVFTAVNLLHSGQTHFD
ALRILPQFETDTREALSLMDRVIAVDQLTS

Native sequence Amino acids M1 – S183 (end) of vOTU.
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 (LEVLFQGP) residues 221 – 228

Cloning sites *Bam*H1 and *Not*1 sites of pGEX6P-1

MRC PPU REAGENTS

Nucleotide Sequence Of Insert

ggatccATGGACTTTCTGCGTTCCCTGGATTGGACACAGGTTATTGCCGGTCAGTATGTGAGCAA
CCCTCGCTTTAACATTTCCGACTATTTCGAGATCGTTCGTCAGCCTGGTGATGGGAATTGCTTCT
ATCACAGCATTGCCGAACTGACAATGCCGAACAAAACCGATCACTCGTATCATTATATCAAACGC
CTGACTGAATCAGCAGCTCGTAAATATTATCAGGAGGAGCCGGAAGCTCGTCTGGTTGGTCTGTC
TCTGGAGGATTATCTGAAACGTATGCTGAGTGACAACGAATGGGGTAGCACTCTGGAAGCCTCTA
TGCTGGCAAAAGAAATGGGCATTACGATCATCATTTGGACCGTTGCCGCCTCCGATGAAGTTGAA
GCCGGGATCAAATTTGGAGATGGCGACGTGTTTACCGCCGTTAATCTGCTGCATAGTGGTCAGAC
CCACTTTGATGCACTGCGTATTCTGCCACAGTTTGAAACAGACACCCGTGAAGCGCTGTCTCTGA
TGGACCGTGTTATTGCCGTGGATCAGCTGACAAGCtaagcggccgc