

## ***MRC PPU REAGENTS***

### **Standard Operating Procedure**

#### **Preparation of VPS26B [1 - 336]**

**Enzyme description:-** VPS26B [1 – 336]

**Clone number:-** DU 62361

**Source:-** Recombinant

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

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Calculated molecular mass:-**

Monoisotopic 65, 936.80 daltons

Average Mass 65, 978.82 daltons

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 6.32

**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

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## Clone Data Sheet

### VPS26B [1 – 336]

**Protein** VPS26B [1 – 336]

**Clone number** DU 62361

**Species** Human

**Accession number** NM\_052875.4

**Tags** N-terminal GST

**Bacterially  
expressed protein**

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFEL  
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAETSMLE  
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN  
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY  
LKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFQGPLGSM**SFFGFGQS**  
**VEVEILLNDAESRKRAEHKTEDGKKEKYFLFYDGETVSGKVSLALKNP**  
**NKRLEHQGIKIEFIGQIELYYDRGNHHEFVSLVKDLARPGEITQSQAF**  
**DFEFTHVEKPYESYTGQNVKLRVFLRATISRRLNDVVKEMDIVVHTLS**  
**TYPELNSSIKMEVGIEDCLHIEFEYNKSKYHLKDVIVGKIYFLLVRIK**  
**IKHMEIDI IKRETTGTGPNVYHENDTIAKYEIMDGAPVRGESIPIRLF**  
**LAGYELTPMRDINKKFSVRYLNLVLI DEEERRYFKQEQEVVLWRKGD**  
**IVRKSMHQAAIASQRFEGTTSLSGEVRTPSQLSDNNCRQ**

**Native sequence** Amino acids M1 – Q336 (end) of human VPS26B.  
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

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### Nucleotide Sequence Of Insert

ggatccATGAGCTTCTTCGGCTTCGGGCAGAGCGTGGAGGTGGAAATCCTTCTGAACGATGCAGA  
GAGTAGGAAGCGGGCCGAGCACAAGACGGAGGACGGGAAGAAGGAGAAATATTTCTTCTTCTACG  
ACGGGGAGACGGTCTCCGGGAAGGTGAGCCTTGCCCTCAAGAACCCCAACAAGCGGCTGGAGCAC  
CAGGGCATCAAGATCGAGTTCATCGGGCAGATCGAACTCTACTACGATCGCGGGAACCACCATGA  
GTTTGTGTCCCTGGTGAAGGACCTGGCCCCGGCCTGGAGAGATCACCCAGTCGCAGGCCTTCGACT  
TTGAGTTTACCCACGTGGAGAAGCCGTATGAGTCCTACACAGGGCAGAATGTGAAGCTACGCTAT  
TTCCTTCGTGCTACCATCAGCCGCCCTCAATGATGTTGTCAAAGAGATGGACATTGTAGTTCA  
CACACTCAGCACATAACCAGAGCTGAACTCTTCCATCAAGATGGAGGTTGGGATTGAGGACTGTC  
TGCACATTGAATTTGAGTACAATAAATCCAAATACCACTTGAAAGATGTCATTGTAGGGAAGATA  
TACTTCCTGCTGGTGAGAATCAAAATCAAGCACATGGAGATAGACATCATCAAGCGAGAAACGAC  
GGGTACAGGCCCAACGTGTACCATGAGAATGACACGATAGCCAAGTACGAGATCATGGACGGGG  
CACCAGTGCAGGAGAGTCCATCCCGATCCGGCTCTTCCTGGCCGGGTATGAGCTCACGCCACC  
ATGCGGGACATCAACAAGAAGTTCTCTGTGCGCTATTACCTCAACCTGGTGCTGATAGACGAGGA  
GGAGCGGCGCTACTTCAAGCAGCAGGAAGTGGTGTGTTGTGGCGGAAGGGTGACATCGTACGGAAGA  
GCATGTCCCACCAGGCGGCCATCGCCTCACAGCGCTTTGAGGGCACCACCTCCCTGGGTGAGGTG  
CGGACCCCAGCCAGCTGTCTGACAACAACCTGCAGGCAGtaggcggccgc