MRC PPU REAGENTS

Standard Operating Procedure

Preparation of VPS26A [1 - 327]

Enzyme description:- VPS26A [1 – 327]

Clone number:- DU 62328

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 64, 952.38 daltons
Average Mass 64, 994.08 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.96

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

VPS26A [1 – 327]

Protein VPS26A [1 – 327]

Clone number DU 62328

Species Human

Accession number O75436-1

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MSFLGGFFG**
PICEIDIVLNDGETRKMAEMKTEDGKVEKHYLFYDGESVSGKVNLAFK
QPGKRLEHQGIRIEFVGQIELFNDKSNTHEFVNLVKELALPGELTQSR
SYDFEFMQVEKPYESYIGANVRLRYFLKVTIVRRLTDLVKEYDLIVHQ
LATYPDVNNSIKMEVGIEDCLHIEFEYNKSKYHLKDVIVGKIYFLLVR
IKIQHMELQLIKKEITGIGPSTTTETETIAKYEIMDGAPVKGESIPIR
LFLAGYDPTPTMRDVNKKFSVRYFLNLVLVDEEDRRYFKQQEIILWRK
APEKLRKQRTNFHQRFESPESQASAEQPEM

Native sequence Amino acids M1 – M327 (end) of human VPS26A.
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

Nucleotide Sequence Of Insert

ggatccATGAGTTTTCTTGGAGGCTTTTTTGGTCCAATTTGTGAGATCGATATTGTTCTTAATGA
TGGGGAAACCAGGAAAATGGCAGAAATGAAAACTGAAGATGGCAAAGTAGAAAAACACTATCTCT
TCTATGACGGAGAATCCGTTTCAGGAAAGGTAAACCTAGCCTTTAAGCAACCTGGAAAGAGGCTA
GAACACCAAGGAATTAGAATTGAATTTGTAGGTCAAATTGAACTTTTCAATGACAAGAGTAATAC
TCATGAATTTGTAAACCTAGTGAAAGAACTAGCCTTACCTGGAGAACTGACTCAGAGCAGAAGTT
ATGATTTTGAATTTATGCAAGTTGAAAAGCCATATGAATCTTACATCGGTGCCAATGTCCGCTTG
AGGTATTTTCTTAAAGTGACAATAGTGAGAAGACTGACAGATTTGGTAAAAGAGTATGATCTTAT
TGTTCACCAGCTTGCCACCTATCCTGATGTTAACAACTCTATTAAGATGGAAGTGGGCATTGAAG
ATTGTCTACATATAGAATTTGAATATAATAAATCAAAGTATCATTTAAAGGATGTGATTGTTGGA
AAAATTTACTTCTTATTAGTAAGAATAAAAATACAACATATGGAGTTACAGCTGATCAAAAAAGA
GATCACAGGAATTGGACCCAGTACCACAACAGAAACAGAAACAATCGCCAAATATGAAATAATGG
ATGGTGCACCAGTAAAAGGTGAATCAATTCCAATAAGGCTATTTTTAGCAGGATATGACCCAACT
CCAACAATGAGAGATGTGAACAAAAAATTTTCAGTAAGGTACTTTTTGAATTTAGTGCTTGTTGA
TGAGGAAGACCGGAGGTACTTCAAACAGCAGGAGATAATTTTATGGAGAAAAGCTCCTGAAAAAC
TGAGGAAACAGAGAACAAACTTTCACCAGCGATTTGAATCTCCAGAATCACAGGCATCTGCCGAA
CAGCCTGAAATGtgagcggccgc