

MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of PPM1J [1 – 505]

Enzyme description:- PPM1J [1 – 505]

Clone number:- DU 68180

Source:- Recombinant

Expression system:- *E.coli*,

Tag:- N-terminal GST

Purification method:- GSH-Sepharose

Calculated molecular mass:-

Monoisotopic 81, 606.43 daltons

Average Mass 81, 658.45 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.47

Purity:- >80 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 2 mM MnCl₂,
0.1 % 2-mercaptoethanol, 0.03 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

PPM1J [1 - 505]

Protein PPM1J [1 - 505]

Clone number DU 68140

Species Human

Accession number NM_005167.5

Tags N-terminal GST

Bacterially expressed PPM1J protein

MSPILGYWKIKGLVQPTRLLEYLEEKYEHHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMATIRYIADKHNMLGGCPKERAETSMLE
GAVLDIRYGVSRIAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY
LKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFGQPLGSMLNRVRS
AHLVSSGGAPPPRPKSPDL PNAASAPPAAPPAPEAPRSPPAKAGSGSATP
AKAVEARASFSRPTFLQLSPGGLRRADDHAGRAVQSPD TGRRLPWST
GYAEVINAGKSRHNEDQACCEVVYVEGRRSVTGVPREPSRGQGLCFYY
WGLFDGHAGGGAEMASRLLRHIREQLKDLVEILQDPSPPPLCLPTT
PGTPDSSDP SHLLGPQSCWSSQKEVSHESLVVGAVENAFQLMDEQMAR
ERRGHQVEGGCCALVVIYLLGKVYVANAGDSRAIIVRNGEIIPMSREF
TPETERQRLQLLGLKPELLGSEFTHLEFPRRVLPKELQRM L YRDQN
MTGWAYKKIELEDLRFPLVCGEKKARVMATIGVTRGLGDHSLKVCSS
TLPIKPFLSCFPEVRVYDLTQYEHCPDDVLVLGTDGLWDVTTDCEVAA
TVDRVLSAYEPNDHSRYTALAQAQALVLGARGTPRDRGWRLPNNKLGSGD
DISVFVIPLGGPGSYS

Native sequence Amino acids M1 – S505 (end) of human PPM1J.
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 - 229

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

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Nucleotide

Sequence Of Insert

ggatccATGCTAAACCGGGTGCGCTCGGCCGTGGCGCACCTGGTGAGC
TCCGGGGGCGCTCCGCCTCCGCGCCCCAAATCCCCGGACCTGCCAAC
GCCGCCTCGGCGCCGCCCGCCGCTCCAGAAGCGCCAGGAGCCCT
CCCGCGAAGGCTGGGAGCGGGAGCGCGACGCCCGCAAGGCTGTTGAG
GCTCGAGCGAGCTTCTCCAGACCGACCTTTCTGCAGCTGAGCCCCGGG
GGGCTGCGACGCGCCGATGACCACGCGGGCCGGGCTGTGCAAAGCCCC
CCGGACACGGGGCCGCCCTGCCCTGGAGCACAGGCTACGCCGAGGTC
ATCAATGCTGGCAAGAGTCGGCACAATGAGGACCAGGCTTGCTGTGAA
GTGGTGTATGTGGAAGGTCGGAGGAGTGTTACAGGAGTACCTAGGGAG
CCTAGCCGAGGCCAGGGACTCTGCTTCTACTACTGGGGCCTATTTGAT
GGGCATGCAGGGGGCGGAGCTGCTGAAATGGCCTCACGGCTCCTGCAT
CGCCATATCCGAGAGCAGCTAAAGGACCTGGTAGAGATACTTCAGGAC
CCTTCGCCACCACCCCTCTGCCTCCCAACCACTCCGGGGACCCAGAT
TCCTCCGATCCCTCTCACTTGCTTGGCCCTCAGTCCTGCTGGTCTTCA
CAGAAGGAAGTGAGCCACGAGAGCCTGGTAGTGGGGGCCGTTGAGAAT
GCCTTCCAGCTCATGGATGAGCAGATGGCCCCGGGAGCGGCGTGGCCAC
CAAGTGGAGGGGGGCTGCTGTGCACTGGTTGTGATCTACCTGCTAGGC
AAGGTGTACGTGGCCAATGCAGGCGATAGCAGGGCCATCATTGTCCGG
AATGGTGAAATCATTTCCAATGTCCCGGGAGTTTACCCCGGAGACTGAG
CGCCAGCGTCTTCAGCTGCTTGGCTTCCCTGAAACCAGAGCTGCTAGGC
AGTGAATTCACCCACCTTGAGTTCCCCCGCAGAGTTCTGCCCAAGGAG
CTGGGGCAGAGGATGTTGTACCGGGACCAGAACATGACCGGCTGGGCC
TACAAAAGATCGAGCTGGAGGATCTCAGGTTTCCTCTGGTCTGTGGG
GAGGGCAAAAAGGCTCGGGTGATGGCCACCATTGGGGTGACCCGAGGC
TTGGGAGACCACAGCCTTAAGGTCTGCAGTTCCACCCTGCCCATCAAG
CCCTTTCTCTCCTGCTTCCCTGAGGTACGAGTGTATGACCTGACACAA
TATGAGCACTGCCCAGATGATGTGCTAGTCCTGGGAACAGATGGCCTG
TGGGATGTCACTACTGACTGTGAGGTAGCTGCCACTGTGGACAGGGTG
CTGTCGGCCTATGAGCCTAATGACCACAGCAGGTATACAGCTCTGGCC
CAAGCTCTGGTCTTGGGGGCCCGGGGTACCCCCGAGACCGTGGCTGG
CGTCTCCCCAACAAAGCTGGGTTCCGGGGATGACATCTCTGTCTTC
GTCATCCCCCTGGGAGGGCCAGGCAGTTACTCCTgagcggccgc