

MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of PPM1D [1 – 605]

Enzyme description:- PPM1D [1 – 605]

Clone number:- DU 32201

Source:- Recombinant

Expression system:- *E.coli*,

Tag:- N-terminal GST

Purification method:- GSH-Sepharose

Calculated molecular mass:-

Monoisotopic 94, 417.99 daltons

Average Mass 94, 478.37 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 8.67

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

Storage temperature:- -70 °C

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Clone Data Sheet

PPM1D [1 - 605]

Protein PPM1D [1 - 605]

Clone number DU 32201

Species Human

Accession number NM_003620.4

Tags N-terminal GST

**Bacterially
expressed PPM1D
protein**

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY
LKSSKYIAWPLQGQWQATFGGGDHPKSDLEVLVFGPLGSPGIPGSTRA
AAMAGLYSLGVSVFS**DQGG****RKYMEDVTQIVVEPEPTAEKPS****PRRSL**
QPLPPRPS**PAALPGGEVSGKPAVAAREARDPLPDAGAS****PAPSRCCRR**
RSSVAFFAVCDGHGGREAAQFAREHLWGF**IKKQKGF****TSSEPAKVCAAI**
RKGF**LACHLAMWKKLAEWPKTMTGLPSTSGTTASVVI****IRGMKMYVAHV**
GDSGVVLGIQDDPKDDFVRAVEVTQDHKPEL**PKERERIEGLGGSVMNK**
SGVNRVVWKRPRLTHNGPVRRSTVIDQIPFLAVARALGDLWSYDF**FSG**
EFVVSPEPDTSVHTLDPQKHKYI**ILGSDGLWNMIPPQDAISMCQDQEE**
KKYLMGEHGQSCAKMLVNRALGRWRQRM**LRADNTSAIVICISPEVDNQ**
GNFTNEDELYLNLTDSPSYNSQETCVMT**PSPCSTPPVKSLEEDPWPRV**
NSKDHIPALVRSNAFSENFLEVSAE**IARENVQGVVIPS****KDPEPLEENC**
AKALTLRIHDSLNNSLPIGLVPTNSTNTVMDQKNLKMSTPGQMK**QEI**
ERTPPTNF**KRTLEESNSGPLM****KKHRRNGLSRSSGAQPASLPTTSQRKN**
SVKLTMRRLRGQKKIGNPLLHQHRKTVCVC

Native sequence Amino acids M1 – C605 (end) of human PPM1D.
Residue M243 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 - 220.

Protease cleavage PreScission (LEVLVFGP) residues 221 - 229

Cloning sites *Not1* sites of pGEX6P-2

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Nucleotide

Sequence Of Insert

gcggccgcgATGGCGGGGCTGTACTCGCTGGGAGTGAGCGTCTTCTCC
GACCAGGGCGGGAGGAAGTACATGGAGGACGTTACTCAAATCGTTGTG
GAGCCCGAACCGACGGCTGAAGAAAAGCCCTCGCCGCGGCGGTGCGTG
TCTCAGCCGTTGCCTCCGCGGCCGTGCGCCGGCCGCCCTTCCCGGCGGC
GAAGTCTCGGGGAAAGGCCAGCGGTGGCAGCCCAGAGGCTCGCGAC
CCTCTCCCGGACGCCGGGGCCTCGCCGGCACCTAGCCGCTGCTGCCGC
CGCCGTTCTCCGTGGCCTTTTTTCGCCGTGTGCGACGGGCACGGCGGG
CGGGAGGCGGCACAGTTTGCCCGGGAGCACTTGTGGGGTTTCATCAAG
AAGCAGAAGGGTTTCACCTCGTCCGAGCCGGCTAAGGTTTGCGCTGCC
ATCCGCAAAGGCTTCTCGCTTGTACCTTGCCATGTGGAAGAACTG
GCGGAATGGCCAAAGACTATGACGGGTCTTCTAGCACATCAGGGACA
ACTGCCAGTGTGGTCATCATTGCGGGCATGAAGATGTATGTAGCTCAC
GTAGGTGACTCAGGGGTGGTTCTTGGAATTCAGGATGACCCGAAGGAT
GACTTTGTGAGAGCTGTGGAGGTGACACAGGACCATAAGCCAGAACTT
CCAAGGAAAGAGAACGAATCGAAGGACTTGGTGGGAGTGTAATGAAC
AAGTCTGGGGTGAATCGTGTAGTTTGGAACGACCTCGACTCACTCAC
AATGGACCTGTTAGAAGGAGCACAGTTATTGACCAGATTCCTTTTCTG
GCAGTAGCAAGAGCACTTGGTGATTTGTGGAGCTATGATTTCTTCAGT
GGTGAATTTGTGGTGTACCTGAACCAGACACAAGTGTCCACACTCTT
GACCCTCAGAAGCACAAAGTATATATATATGTTGGGAGTGATGGACTTTGG
AATATGATTCCACCACAAGATGCCATCTCAATGTGCCAGGACCAAGAG
GAGAAAAAATACCTGATGGGTGAGCATGGACAATCTTGTGCCAAAATG
CTTGTGAATCGAGCATTGGGCCGCTGGAGGCAGCGTATGCTCCGAGCA
GATAACACTAGTGCCATAGTAATCTGCATCTCTCCAGAAGTGGACAAT
CAGGGAAACTTTACCAATGAAGATGAGTTATACCTGAACCTGACTGAC
AGCCCTTCTATAATAGTCAAGAAACCTGTGTGATGACTCCTTCCCA
TGTTCTACACCACCAGTCAAGTCACTGGAGGAGGATCCATGGCCAAGG
GTGAATTCTAAGGACCATATACCTGCCCTGGTTCGTAGCAATGCCTTC
TCAGAGAATTTTTTAGAGGTTTTAGCTGAGATAGCTCGAGAGAATGTC
CAAGGTGTAGTCATACCTCAAAAAGATCCAGAACCACTTGAAGAAAAT
TGCGCTAAAGCCCTGACTTTAAGGATACATGATTCTTTGAATAATAGC
CTTCCAATTGGCCTTGTGCCTACTAATTCAACAAACACTGTCATGGAC
CAAAAAAATTTGAAGATGTCAACTCCTGGCCAAATGAAAGCCCAAGAA
ATTGAAAGAACCCCTCCAACAACTTTAAAAGGACATTAGAAGAGTCC
AATTCTGGCCCCCTGATGAAGAAGCATAGACGAAATGGCTTAAGTCGA
AGTAGTGGTGCTCAGCCTGCAAGTCTCCCCACAACCTCACAGCGAAAG
AACTCTGTAAACTCACCATGCGACGCAGACTTAGGGGCCAGAAGAAA
ATTGAAATCCTTTACTTCATCAACACAGGAAAACCTGTTTGTGTTTGC
tgagcggccgc

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