

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active PPM1 beta isoform 1 [2 - 479] (PP2C beta isoform 1)

<u>Enzyme description:-</u>	PPM1 beta isoform 1 [2 - 479]
<u>Clone number:-</u>	DU 3021
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i>
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	GSH Sepharose
<u>Expression level:-</u>	5-10 mg/L
<u>Calculated molecular mass:-</u>	79, 658 daltons
<u>Purity:-</u>	>80 %
<u>Activation protocol:-</u>	Constitutively active

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 2 mM MnCl₂, 0.03 % Brij 35, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 50 % glycerol, 1 mM benzamidine and 0.1 mM PMSF

Storage temperature:- -20 °C

Assay:- Standard phosphatase assay

Assay buffer:-

50 mM Tris-HCl pH 7.5, 2 mM MnCl₂, 0.03 % Brij 35, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol

Substrate:-

6 μM ³²P labelled casein (phosphorylated by PKA)

Specific activity range:- To be determined

Division of Signal Transduction Therapy

Clone Data Sheet

PPM1 beta isoform 1 [2 – 479]

Protein PPM1 beta isoform 1 [2 – 479]

Clone number DU 3021

Species Human

Accession number AAH64381

Tags N-terminal GST

Bacterially expressed protein MSPILGYWKIKGLVQPTRLLEYLEEKYEHLIERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAESMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY
LKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFGQPLGSPEFGAFLDK
PKTEKHNAHGAGNGLRYGLSSMQGWRVEMEDAHTAVVGI PHGLEDWSF
FAVYDGHAGSRVANYCSTHLLHEHITTNEFDRAAGKSGSALELSVENVK
NGIRTGFLKIDEYMRNFSDLRNGMDRSGSTAVGVMI SPKHIYFINCGD
SRAVLYRNGQVCFSTQDHPKCNPREKERIQNAGGSVMIQRVNGSLAVS
RALGDYDYKCVDGKGPTEQLVSPPEVYEILRAEDEFIILACDGIWD
VMSNEELCEYVKSRLVSDDLENCNWWVDTCLHKGSRDNMSIVLVCF
SNAPKVSDEAVKKDSELDKHLESRVVEIMEKSGEEMPDLAHVMIILS
AENIPNLPPGGGLAGKRNVIEAVYSRLNPHRESDGASDEAEESGSQGK
LVEALRQMRINHRGNRYRQLLEEMLT SYRLAKVEGEE SPAEPAATATSS
NSDAGNPVTMQESHTESESGLAELDSSNEDAGTKMSGEKI

Native sequence Amino acids G2 – I479 (end) of human PPM1 beta isoform 1.
Residue G235 of the fusion protein is equivalent to G2 of the native
enzyme The GST tag is located at residues 1 - 220.

Protease cleavage PreScission (LEVLFQGPL) at residues 221 - 229

Cloning sites *EcoRI* and *NotI* sites of pGEX-6P-1

Division of Signal Transduction Therapy

Nucleotide
sequence of insert

gaattcGGTGCAATTTTTGGATAAACCCAAAACCTGAAAAACATAATGCT
CATGGTGCTGGGAATGGTTTACGTTATGGCCTGAGCAGCATGCAAGGA
TGGAGAGTGGAATGGAAGATGCACACACAGCTGTTGTAGGTATTCCCT
CACGGCTTGGAAGACTGGTCATTTTTTGCAGTTTATGATGGTCATGCT
GGATCCCGAGTGGCAAATTACTGCTCAACACATTTATTAGAACACATC
ACTACTAACGAAGACTTTAGGGCAGCTGGAAAATCAGGATCTGCTCTT
GAGCTTTCAGTGGAAAATGTTAAGAATGGTATCAGAACTGGATTTTTTG
AAAATTGATGAATACATGCGTAACTTTTCAGACCTCAGAAACGGGATG
GACAGGAGTGGTTCAACTGCAGTGGGAGTTATGATTTACCTAAGCAT
ATCTACTTTATCAACTGTGGTGATTACAGTGCTGTTCTGTATAGGAAT
GGACAAGTCTGCTTTTCTACCCAGGATCACAAACCTTGCAATCCAAGG
GAAAAGGAGCGAATCCAAAATGCAGGAGGCAGCGTGATGATACAACGT
GTTAATGGTTCATTAGCAGTATCTCGTGCTCTGGGGGACTATGATTAC
AAGTGTGTTGATGGCAAGGGCCCAACAGAACAACTTGTTTCTCCAGAG
CCTGAGGTTTATGAAATTTTAAGAGCAGAAGAGGATGAATTTATCATC
TTGGCTTGTGATGGGATCTGGGATGTTATGAGTAATGAGGAGCTCTGT
GAATATGTTAAATCTAGGCTTGAGGTATCTGATGACCTGGAAAATGTG
TGCAATTGGGTAGTGGACACTTGTTTACACAAGGGAAGTCGAGATAAC
ATGAGTATTGTAAGTGTGCTTTTCAAATGCTCCCAAGGTCTCAGAT
GAAGCGGTGAAAAAAGATTAGAGTTGGATAAGCACTTGAATCACGG
GTTGAAGAGATTATGGAGAAGTCTGGCGAGGAAGGAATGCCTGATCTT
GCCCATGTCATGCGCATCTTGTCTGCAGAAAATATCCCAAATTTGCCT
CCTGGGGGAGGTCTTGCTGGCAAGCGTAATGTTATTGAAGCTGTTTAT
AGTAGACTGAATCCACATAGAGAAAGTGATGGGGCCTCCGATGAAGCA
GAGGAAAGTGGATCACAGGGAAAATTGGTGGAAAGCTCTCAGGCAAATG
AGAATTAATCATAGGGGAAACTACCGACAACCTTCTGGAGGAGATGCTG
ACTAGTTACAGGCTAGCTAAAGTAGAGGGAGAAGAAAGCCCTGCTGAA
CCAGCTGCCACAGCTACTTCTTGAACAGTGATGCTGGAAACCCAGTG
ACAATGCAGGAAAGCCATACTGAATCAGAAAGTGGTCTTGCTGAATTA
GACAGCTCTAATGAAGATGCAGGGACAAAGATGAGTGTTGAAAAATA
tgagcggccgc