Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of PPM1H [1 – 514]

Enzyme description:- PPM1H [1 – 514]

Clone number:- DU 62941

Source:- Recombinant

Expression system:- *E.coli*,

Tag:- N-terminal GST

Purification method:- GSH-Sepharose

Calculated molecular mass:-

Monoisotopic	83, 219.27 daltons
Average Mass	83, 272.18 daltons

[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.01

Purity:- >80 %

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 2 mM $MnCl_2$,
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

PPM1H [1 - 514]

Protein	PPM1H [1 - 514]
Clone number	DU 62941
Species	Human
Accession number	Q9ULR3.2
Tags	N-terminal GST
Bacterially expressed PPM1H protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MLTRVKSAV** **ANFMGGIMAGSSGSEHGGGSCGGSDLPLRFPYGRPEFLGLSQDEVECS** **ADHIARPILILKETRRLPWATGYAEVINAGKSTHNEDQASCEVLTVKK** **KAGAVTSTPNRNSSKRRSSLPNGEGLQLKENSESEGVSCHYWSLFDGH** **AGSGAAVVASRLLQHHITEQLQDIVDILKNSAVLPPTCLGEEPENTPA** **NSRTLTRAASLRGGVGAPGSPSTPPTRFFTEKKIPHECLVIGALESAF** **KEMDLQIERERSSYNISGGCTALIVICLLGKLYVANAGDSRAIIIRNG** **EIIPMSSEFTPETERQRLQYLAFMQPHLLGNEFTHLEFPRRVQRKELG** **KKMLYRDFNMTGWAYKTIEDEDLKFPLIYGEGKKARVMATIGVTRGLG** **DHDLKVHDSNIYIKPFLSSAPEVRIYDLSKYDHGSDDVLILATDGLWD** **VLSNEEVAEAITQFLPNCDPDDPHRYTLAAQDLVMRARGVLKDRGWRI** **SNDRLGSGDDISVYVIPLIHGNKLS**
Native sequence	Amino acids M1 – S514 (end) of human PPM1H. 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*1 sites of pGEX6P1

Nucleotide Sequence Of Insert

ggatccATGCTCACTCGAGTGAAATCTGCCGTGGCCAATTTCATGGGC
GGCATCATGGCTGGCAGCTCAGGCTCCGAGCACGGCGGCGGCAGCTGC
GGAGGCTCGGACCTGCCCCTGCGTTTCCCCTACGGGCGGCCAGAGTTC
CTGGGGCTGTCTCAGGACGAGGTGGAGTGCAGCGCCGACCACATCGCC
CGCCCCATCCTCATCCTCAAGGAGACTCGGCGGCTGCCCTGGGCCACT
GGCTACGCAGAGGTTATCAATGCCGGGAAGAGCACACACAATGAAGAC
CAAGCCAGCTGTGAGGTGCTCACTGTGAAGAAGAAGGCAGGGGCCGTG
ACCTCAACCCCAAACAGGAACTCATCCAAGAGACGGTCCTCCCTTCCC
AATGGGGAAGGGCTGCAGCTGAAGGAGAACTCGGAATCCGAGGGTGTT
TCCTGCCACTATTGGTCGCTGTTTGACGGGCACGCGGGGTCCGGGGCC
GCGGTGGTGGCGTCACGCCTGCTGCAGCACCACATCACGGAGCAGCTG
CAGGACATCGTGGACATCCTGAAGAACTCCGCCGTCCTGCCCCCTACC
TGCCTGGGGGAGGAGCCTGAGAACACGCCCGCCAACAGCCGGACTCTG
ACCCGGGCAGCCTCCCTGCGCGGAGGGGTGGGGGCCCCGGGCTCCCCC
AGCACGCCCCCCACACGCTTCTTTACCGAGAAGAAGATTCCCCATGAG
TGCCTGGTCATCGGAGCGCTTGAAAGTGCATTCAAGGAAATGGACCTA
CAGATAGAACGAGAGAGGAGTTCATATAATATATCTGGTGGCTGCACG
GCCCTCATTGTGATTTGCCTTTTGGGGAAGCTGTATGTTGCAAATGCT
GGGGATAGCAGGGCCATAATCATCAGAAATGGAGAAATTATCCCCATG
TCTTCAGAATTTACCCCCGAGACGGAGCGCCAGCGACTTCAGTACCTG
GCATTCATGCAGCCTCACTTGCTGGGAAATGAGTTCACACATTTGGAG
TTTCCAAGGAGAGTACAGAGAAAGGAGCTTGGAAAGAAGATGCTCTAC
AGGGACTTTAATATGACAGGCTGGGCATACAAAACCATTGAGGATGAG
GACTTGAAGTTCCCCCTTATATATGGAGAAGGCAAGAAGGCCCGGGTA
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GTGCATGACTCCAACATCTACATTAAACCATTCCTGTCTTCAGCTCCA
GAGGTAAGAATCTACGATCTTTCAAAATATGATCATGGATCAGATGAT
GTGCTGATCTTGGCCACTGATGGACTCTGGGACGTTTTATCAAATGAA
GAAGTAGCAGAAGCAATCACTCAGTTTCTTCCTAACTGTGATCCAGAT
GATCCTCACAGGTACACACTGGCAGCTCAGGACCTGGTGATGCGTGCC
CGGGGTGTGCTGAAGGACAGAGGATGGCGGATATCTAATGACCGACTG
GGCTCAGGAGACGACATTTCTGTATATGTCATTCCTTTAATACATGGA
AACAAGCTGTCAtgagcggccgc

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