

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of PPM1H [1 – 514]

<u>Enzyme description:-</u>	PPM1H [1 – 514]
<u>Clone number:-</u>	DU 62835
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i> ,
<u>Tag:-</u>	N-terminal His(6) - SUMO
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose
<u>Calculated molecular mass:-</u>	
Monoisotopic	68, 399.49 daltons
Average Mass	68, 442.44 daltons
	[cysteines reduced, methionines have not been oxidised]
<u>Theoretical pI:-</u>	6.08
<u>Purity:-</u>	>80 %
<u>Enzyme storage buffer:-</u>	50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.03 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF
<u>Storage temperature:-</u>	-70 °C

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Clone Data Sheet

PPM1H [1 - 514]

Protein PPM1H [1 - 514]

Clone number DU 62835

Species Human

Accession number Q9ULR3.2

Tags N-terminal His(6) + SUMO

Bacterially expressed PPM1H protein MGHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFVKVMT
THLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIE
VYQEQTGGMLTRVKS AVANFMGGIMAGSSGSEHGGGSCGSDLPLRFP
YGRPEFLGLSQDEVECSADHIARPILILKETRRLPWATGYAEVINAGK
STHNEDQASCEVLTVKKKAGAVTSTPNRNSSKRRSSLPNGEGLQLKEN
SESEGVSCHYWSLFDGHAGSGAAVVASRLLQHHITEQLQDIVDILKNS
AVLPPTCLGEEPENTPANSRTLTRAASLRGGVGAPGSPSTPPTRFFTE
KKIPHECLVIGALES AFKEMDLQIERERSSYNISGGCTALIVICLLGK
LYVANAGDSRAIIIRNGEIIIPMSSEFTPETERQRLQYLAFMOPHLLGN
EFTHLEFPRRVQRKELGKKMLYRDFNMTGWAYKTIEDEDLKFPLIYGE
GKKARVMATIGVTRGLGDHDLKVHDSNIYIKPFLSSAPEVRIYDLSKY
DHGSDDVLILATDGLWDVLSNEEVAEAITQFLPNCDDPHRYTLAAQ
DLVMRARGVLKDRGWRI SNDRLGSGDDISVYVIPLIHGNKLS

Native sequence Amino acids M1 – S514 (end) of human PPM1H.
Residue M105 of the fusion protein is equivalent to M1 of the native enzyme. The His(6) tag is located at residues 2 – 7.

Protease cleavage SENP1 cleavage of SUMO:
(SDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFVKVMTT
HLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGME
EEDVIEVYQEQTGG) residues 9 - 104

Cloning sites *Bam*H1 and *Not*1 sites of pET15b His6-SUMO

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Complete
Nucleotide
Sequence

ATGGGTCATCATCACCATCACCATTCTGACCAGGAGGCAAAACCTTCA
ACTGAGGACTTGGGGGATAAGAAGGAAGGTGAATATATTAAACTCAA
GTCATTGGACAGGATAGCAGTGAGATTCACCTCAAAGTGAAAATGACA
ACACATCTCAAGAACTCAAAGAATCATACTGTCAAAGACAGGGTGT
CCAATGAACTCACTCAGGTTTCTCTTTGAGGGTCAGAGAATTGCTGAT
AATCATACTCCAAAAGAACTGGGAATGGAGGAAGAAGATGTGATTGAA
GTTTATCAGGAACAAACGGGGGAATGCTCACTCGAGTGAAATCTGCC
GTGGCCAATTTTCATGGGCGGCATCATGGCTGGCAGCTCAGGCTCCGAG
CACGGCGGCGGCAGCTGCGGAGGCTCGGACCTGCCCTGCGTTTCCCC
TACGGGCGGCCAGAGTTCTGGGGCTGTCTCAGGACGAGGTGGAGTGC
AGCGCCGACCACATCGCCCGCCCCATCCTCATCCTCAAGGAGACTCGG
CGGCTGCCCTGGGCCACTGGCTACGCAGAGGTTATCAATGCCGGGAAG
AGCACACACAATGAAGACCAAGCCAGCTGTGAGGTGCTCACTGTGAAG
AAGAAGGCAGGGGCCGTGACCTCAACCCAAACAGGAACTCATCCAAG
AGACGGTCTTCCCTTCCAATGGGGAAGGGCTGCAGCTGAAGGAGAAC
TCGGAATCCGAGGGTGTTCCTGCCACTATTGGTTCGCTGTTTGACGGG
CACGCGGGGTCCGGGGCCGCGGTGGTGGCGTCACGCCTGCTGCAGCAC
CACATCACGGAGCAGCTGCAGGACATCGTGGACATCCTGAAGAACTCC
GCCGTCTGCCCTTACCTGCCTGGGGGAGGAGCCTGAGAACACGCC
GCCAACAGCCGACTCTGACCCGGGCAGCCTCCCTGCGCGGAGGGGTG
GGGGCCCCGGGCTCCCCAGCACGCCCCCACACGCTTCTTTACCGAG
AAGAAGATTCCCCATGAGTGCCTGGTTCATCGGAGCGCTTGAAAGTGCA
TTCAAGGAAATGGACCTACAGATAGAACGAGAGAGGAGTTCATATAAT
ATATCTGGTGGCTGCACGGCCCTCATTGTGATTTGCCTTTTGGGGAAG
CTGTATGTTGCAAAATGCTGGGGATAGCAGGGCCATAATCATCAGAAAT
GGAGAAATTATCCCCATGTCTTCAGAATTTACCCCCGAGACGGAGCGC
CAGCGACTTCAGTACCTGGCATTTCATGCAGCCTCACTTGGTGGAAAT
GAGTTCACACATTTGGAGTTTCCAAGGAGAGTACAGAGAAAGGAGCTT
GGAAAGAAGATGCTCTACAGGGACTTTAATATGACAGGCTGGGCATAC
AAAACCATTGAGGATGAGGACTTGAAGTTCCCCCTTATATATGGAGAA
GGCAAGAAGGCCCGGGTAATGGCAACTATTGGAGTGACCAGGGGACTT
GGGGACCATGACCTGAAGGTGCATGACTCCAACATCTACATTAAACCA
TTCTGTCTTCAGCTCCAGAGGTAAGAATCTACGATCTTTCAAATAT
GATCATGGATCAGATGATGTGCTGATCTTGGCCACTGATGGACTCTGG
GACGTTTTATCAAATGAAGAAGTAGCAGAAGCAATCACTCAGTTTCTT
CCTAACTGTGATCCAGATGATCCTCACAGGTACACACTGGCAGCTCAG
GACCTGGTATGCGTGCCCGGGGTGTGCTGAAGGACAGAGGATGGCGG
ATATCTAATGACCGACTGGGCTCAGGAGACGACATTTCTGTATATGTC
ATTCCTTTAATACATGGAAACAAGCTGTCAtgagcggccgc

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