

MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of PPM1H H153R [1 – 514]

Enzyme description:- PPM1H H153R [1 – 514]
Clone number:- DU 68071
Source:- Recombinant
Expression system:- *E.coli*,
Tag:- N-terminal His(6) - SUMO

Purification method:- Ni²⁺-NTA agarose

Calculated molecular mass:-

Monoisotopic 68, 418.54 daltons
Average Mass 68, 461.48 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.11

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

PPM1H H153R [1 - 514]

<u>Protein</u>	PPM1H H153R [1 - 514]
<u>Clone number</u>	DU 68071
<u>Species</u>	Human
<u>Accession number</u>	Q9ULR3.2
<u>Tags</u>	N-terminal His(6) + SUMO
<u>Bacterially expressed PPM1H protein</u>	MGHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMT THLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIE VYQEQTGGMLTRVKSAVANFMGGIMAGSSGSEHGGGSCGSDLPLRFP YGRPEFLGLSQDEVECSADHIARPILILKETRRLPWATGYAEVINAGK STHNEDQASCEVLTVKKKAGAVTSTPNRNSSKRRSSLPNGEGLQLKEN SESEGVSCHYWSLFDGRAGSGAAVVASRLLQHHITEQLQDIVDILKNS AVLPPTCLGEEPENTPANSRTLTRAASLRGGVGAPGSPSTPPTRFFTE KKIPHECLVIGALESAFKEMDLQIERERSSYNISGGCTALIVICLLGK LYVANAGDSRAIIIRNGEIIPMSSEFTPETERQRLQYLAFMOPHLLGN EFTHLEFPRRVQRKELGKKMLYRDFNMTGWAYKTIEDEDLKFPLIYGE GKKARVMATIGVTRGLGDHDLKVHDSNIYIKPFLSSAPEVRIYDLSKY DHGSDDVLILATDGLWDVLSNEEVAEAITQFLPNCDPDDPHRYTLAAQ DLVMRARGVLKDRGWRISNDRLGSGDDISVYVIPLIHGNKLS
<u>Native sequence</u>	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. The enzyme has a H153R mutation. Residue H153 is equivalent to R257 of the fusion protein.
<u>Protease cleavage</u>	SEN1 cleavage of SUMO: (SDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTT HLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGME EEDVIEVYQEQTGG) residues 9 - 104

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Cloning sites

*Bam*H1 and *Not*I sites of pET15b His6-SUMO

Complete Nucleotide Sequence

ATGCTCACTCGAGTGAAATCTGCCGTGGCCAATTTTCATGGGCGGCATC
ATGGCTGGCAGCTCAGGCTCCGAGCACGGCGGGCGGCAGCTGCGGAGGC
TCGGACCTGCCCCCTGCGTTTCCCCCTACGGGCGGCCAGAGTTCCTGGGG
CTGTCTCAGGACGAGGTGGAGTGCAGCGCCGACCACATCGCCCGCCCC
ATCCTCATCCTCAAGGAGACTCGGCGGCTGCCCTGGGCCACTGGCTAC
GCAGAGGTTATCAATGCCGGGAAGAGCACACACAATGAAGACCAAGCC
AGCTGTGAGGTGCTCACTGTGAAGAAGAAGGCAGGGGGCCGTGACCTCA
ACCCCAAATAGGAATCATCCAAGAGACGGTCCTCCCTTCCCAATGGG
GAAGGGCTGCAGCTGAAGGAGAACTCGGAATCCGAGGGTGTTTCCTGC
CACTATTGGTCGCTGTTTGACGGGCGCGCGGGGTCCGGGGCCGCGGTG
GTGGCGTCACGCCTGCTGCAGCACCACATCACGGAGCAGCTGCAGGAC
ATCGTGGACATCCTGAAGAACTCCGCCGTCTTGCCCCCTACCTGCCTG
GGGGAGGAGCCTGAGAACACGCCCGCCAACAGCCGGACTCTGACCCGG
GCAGCCTCCCTGCGCGGAGGGGTGGGGGCCCCGGGCTCCCCCAGCACG
CCCCCACACGCTTCTTTACCGAGAAGAAGATTCCCCATGAGTGCCTG
GTCATCGGAGCGCTTGAAAGTGCATTCAAGGAAATGGACCTACAGATA
GAACGAGAGAGGAGTTCATATAATATATCTGGTGGCTGCACGGCCCTC
ATTGTGATTTGCCTTTTGGGGAAGCTGTATGTTGCAAATGCTGGGGAT
AGCAGGGCCATAATCATCAGAAATGGAGAAATTATCCCCATGTCTTCA
GAATTTACCCCCGAGACGGAGCGCCAGCGACTTCAGTACCTGGCATTC
ATGCAGCCTCACTTGCTGGGAAATGAGTTCACACATTTGGAGTTTCCA
AGGAGAGTACAGAGAAAGGAGCTTGGAAGAAGATGCTCTACAGGGAC
TTTAATATGACAGGCTGGGCATACAAAACCATTGAGGATGAGGACTTG
AAGTTCCCCCTTATATATGGAGAAGGCAAGAAGGCCCGGGTAATGGCA
ACTATTGGAGTGACCAGGGGACTTGGGGACCATGACCTGAAGGTGCAT
GACTCCAACATCTACATTAAACCATTTCCTGTCTTCAGCTCCAGAGGTA
AGAATCTACGATCTTTCAAATATGATCATGGATCAGATGATGTGCTG
ATCTTGGCCACTGATGGACTCTGGGACGTTTTATCAAATGAAGAAGTA
GCAGAAGCAATCACTCAGTTTCTTCCTAACTGTGATCCAGATGATCCT
CACAGGTACACACTGGCAGCTCAGGACCTGGTGATGCGTGCCCGGGGT
GTGCTGAAGGACAGAGGATGGCGGATATCTAATGACCGACTGGGCTCA
GGAGACGACATTTCTGTATATGTCATTTCCTTTAATACATGGAAACAAG
CTGTCAtgagcggccgcg

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