*MRC PPU Reagents and Services*

# Standard Operating Procedure

# Preparation of PPM1H H153D [1 – 514]

**Enzyme description:-** PPM1H H153D [1 – 514]

**Clone number:-** DU 68104

**Source:-** Recombinant

**Expression system:-** *E.coli*,

**Tag:-** N-terminal His(6) - SUMO

**Purification method**:- $Ni^{2+}$-NTA agarose

**Calculated molecular mass:-**

| | |
|---|---|
| Monoisotopic | 68, 377.46 daltons |
| Average Mass | 68, 420.38 daltons |

[cysteines reduced, methionines have not been oxidised

**Theoretical pI:-** 5.99

**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 H153D [1 - 514]

**Protein** PPM1H H153D [1 - 514]

**Clone number** DU 68104

**Species** Human

**Accession number** Q9ULR3.2

**Tags** N-terminal His(6) + SUMO

**Bacterially expressed PPM1H protein**

MGHHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMT
THLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIE
VYQEQTGG**MLTRVKSAVANFMGGIMAGSSGSEHGGGSCGGSDLPLRFP**
**YGRPEFLGLSQDEVECSADHIARPILILKETRRLPWATGYAEVINAGK**
**STHNEDQASCEVLTVKKKAGAVTSTPNRNSSKRRSSLPNGEGLQLKEN**
**SESEGVSCHYWSLFDGDAGSGAAVVASRLLQHHITEQLQDIVDILKNS**
**AVLPPTCLGEEPENTPANSRTLTRAASLRGGVGAPGSPSTPPTRFFTE**
**KKIPHECLVIGALESAFKEMDLQIERERSSYNISGGCTALIVICLLGK**
**LYVANAGDSRAIIIRNGEIIPMSSEFTPETERQRLQYLAFMQPHLLGN**
**EFTHLEFPRRVQRKELGKKMLYRDFNMTGWAYKTIEDEDLKFPLIYGE**
**GKKARVMATIGVTRGLGDHDLKVHDSNIYIKPFLSSAPEVRIYDLSKY**
**DHGSDDVLILATDGLWDVLSNEEVAEAITQFLPNCDPDDPHRYTLAAQ**
**DLVMRARGVLKDRGWRISNDRLGSGDDISVYVIPLIHGNKLS**

**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.

The enzyme has a H153D mutation. Residue H153 is equivalent to D257 of the fusion protein.

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

**Cloning sites**

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

**Complete Nucleotide Sequence**

ATGCTCACTCGAGTGAAATCTGCCGTGGCCAATTTCATGGGCGGCATC
ATGGCTGGCAGCTCAGGCTCCGAGCACGGCGGCGGCAGCTGCGGAGGC
TCGGACCTGCCCCTGCGTTTCCCCTACGGGCGGCCAGAGTTCCTGGGG
CTGTCTCAGGACGAGGTGGAGTGCAGCGCCGACCACATCGCCCGCCCC
ATCCTCATCCTCAAGGAGACTCGGCGGCTGCCCTGGGCCACTGGCTAC
GCAGAGGTTATCAATGCCGGGAAGAGCACACACAATGAAGACCAAGCC
AGCTGTGAGGTGCTCACTGTGAAGAAGAAGGCAGGGGCCGTGACCTCA
ACCCCAAATAGGAACTCATCCAAGAGACGGTCCTCCCTTCCCAATGGG
GAAGGGCTGCAGCTGAAGGAGAACTCGGAATCCGAGGGTGTTTCCTGC
CACTATTGGTCGCTGTTTGACGGGGACGCGGGGTCCGGGGCCGCGGTG
GTGGCGTCACGCCTGCTGCAGCACCACATCACGGAGCAGCTGCAGGAC
ATCGTGGACATCCTGAAGAACTCCGCCGTCCTGCCCCCTACCTGCCTG
GGGGAGGAGCCTGAGAACACGCCCGCCAACAGCCGGACTCTGACCCGG
GCAGCCTCCCTGCGCGGAGGGGTGGGGGCCCCGGGCTCCCCCAGCACG
CCCCCCACACGCTTCTTTACCGAGAAGAAGATTCCCCATGAGTGCCTG
GTCATCGGAGCGCTTGAAAGTGCATTCAAGGAAATGGACCTACAGATA
GAACGAGAGAGGAGTTCATATAATATATCTGGTGGCTGCACGGCCCTC
ATTGTGATTTGCCTTTTGGGGAAGCTGTATGTTGCAAATGCTGGGGAT
AGCAGGGCCATAATCATCAGAAATGGAGAAATTATCCCCATGTCTTCA
GAATTTACCCCCGAGACGGAGCGCCAGCGACTTCAGTACCTGGCATTC
ATGCAGCCTCACTTGCTGGGAAATGAGTTCACACATTTGGAGTTTCCA
AGGAGAGTACAGAGAAAGGAGCTTGGAAAGAAGATGCTCTACAGGGAC
TTTAATATGACAGGCTGGGCATACAAAACCATTGAGGATGAGGACTTG
AAGTTCCCCCTTATATATGGAGAAGGCAAGAAGGCCCGGGTAATGGCA
ACTATTGGAGTGACCAGGGGACTTGGGGACCATGACCTGAAGGTGCAT
GACTCCAACATCTACATTAAACCATTCCTGTCTTCAGCTCCAGAGGTA
AGAATCTACGATCTTTCAAAATATGATCATGGATCAGATGATGTGCTG
ATCTTGGCCACTGATGGACTCTGGGACGTTTTATCAAATGAAGAAGTA
GCAGAAGCAATCACTCAGTTTCTTCCTAACTGTGATCCAGATGATCCT
CACAGGTACACACTGGCAGCTCAGGACCTGGTGATGCGTGCCCGGGGT
GTGCTGAAGGACAGAGGATGGCGGATATCTAATGACCGACTGGGCTCA
GGAGACGACATTTCTGTATATGTCATTCCTTTAATACATGGAAACAAG
CTGTCAtgagcggccgc