

## *MRC PPU Reagents and Services*

### **Standard Operating Procedure**

#### **Preparation of PPM1M [1 – 459]**

<b><u>Enzyme description:-</u></b>	PPM1M [1 – 459]
<b><u>Clone number:-</u></b>	DU 68141
<b><u>Source:-</u></b>	Recombinant
<b><u>Expression system:-</u></b>	<i>E.coli</i> ,
<b><u>Tag:-</u></b>	N-terminal His(6) - SUMO
<b><u>Purification method:-</u></b>	Cobalt agarose
<b><u>Calculated molecular mass:-</u></b>	
Monoisotopic	63, 090.70 daltons
Average Mass	63, 130.30 daltons
	[cysteines reduced, methionines have not been oxidised]
<b><u>Theoretical pI:-</u></b>	5.69
<b><u>Purity:-</u></b>	>80 %
<b><u>Enzyme storage buffer:-</u></b>	50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 2 mM MnCl <sub>2</sub> , 0.1 % 2-mercaptoethanol, 0.03 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF
<b><u>Storage temperature:-</u></b>	-70 °C

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**Clone Data Sheet**

**PPM1M [1 - 459]**

**Protein** PPM1M [1 - 459]

**Clone number** DU 68141

**Species** Human

**Accession number** NM\_144641.4

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

**Bacterially  
expressed PPM1M  
protein**

MGHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMT  
THLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIE  
VYQEQTGGMSAGWFRRRFLPGEPLPAPRPPGPHASVPYRRPRFLRGS  
SSSPGAADASRRPDSRPVRSARGRTL PWNAGYAEI INAEKSEFNEDQ  
AACGKLCIRRCEFGAEEEWLTLCPPEEFLTGHYWALFDGHGGPAAAILA  
ANTLHSCLRRLQLEAVVEGLVATQPPMHLNGRCICPSDPQFVEEKGIRA  
EDLVIGALES AFQECDEVIGRELEASGQMGCTALVAVSLQGLYMAN  
AGDSRAILVRRDEIRPLSFEFTPETERQRIQQLAFVYPELLAGEFTRL  
EFPRLKGDLLGQKVLFRDHHMSGWSYKRVEKSDLKYPLIHGQGRQAR  
LLGTLAVSRGLGDHQLRVLD TNIQLPFLLSVPQVTVLVDVDQLELQED  
DVVVMATDGLWDVLSNEQVAWLVR SFLPGNQEDPHRF SKLAQMLIHST  
QKEDSLTEEGQVSYDDVSVFVIPLHSQGQESSDH

**Native sequence** Amino acids M1 – H459 (end) of human PPM1M.  
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:  
(SDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTT  
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  
GTTTATCAGGAACAAACGGGGGGAATGTCCGCCGGCTGGTTCCGGCGC  
CGCTTCCTGCCTGGGGAGCCGCTCCCCGCGCCGCTCCACCTGGGCCG  
CATGCCAGCCCCGTGCCCTACCGACGGCCCCGCTTCCTTCGCGGCTCC  
AGCTCCAGCCCCGGGGCGGCCGACGCCTCGCGCCGCCAGACTCCCGG  
CCCGTGCGCAGCCCCGCACGAGGACGCACGCTACCCTGGAATGCAGGC  
TACGCCGAGATTATCAATGCAGAGAAATCTGAATTCAATGAGGATCAA  
GCCGCCTGTGGGAAGCTGTGCATCCGGAGATGTGAGTTTGGGGCTGAA  
GAAGAGTGGCTGACCCTGTGCCAGAGGAGTTCCTGACAGGCCATTAC  
TGGGCACTGTTTCGATGGGCACGGCGGTCTGCAGCAGCCATCTTGGCT  
GCCAACACCCTGCACTCCTGCTTGCGCCGGCAGCTGGAGGCCGTGGTG  
GAAGGCTTGGTGGCCACTCAGCCCCCATGCACCTCAATGGCCGCTGC  
ATCTGCCCCAGTGACCCTCAGTTTGTGGAGAAAAGGGCATCAGGGCA  
GAAGACTTGGTGATCGGGGCATTGGAGAGTGCTTTTCAAGGAATGTGAT  
GAGGTGATCGGGCGGGAGCTGGAGGCCTCAGGCCAGATGGGCGGCTGC  
ACAGCCCTGGTGGCTGTGTCCCTGCAGGGAAAGCTGTACATGGCCAAT  
GCTGGGGATAGCAGGGCCATCTTGGTGCAGGAGAGATGAGATACGGCCA  
CTGAGCTTCGAGTTCACCCAGAGACTGAGCGGCAGCGTATTCAGCAG  
CTGGCCTTTGTCTATCCTGAGCTTCTGGCTGGTGAGTTCACCCGACTG  
GAGTTCCCTCGGCGGCTGAAGGGGGATGACTTGGGACAGAAGGTTTTG  
TTCAGGGATCACACATGAGTGGCTGGAGCTACAAACGTGTGGAGAAA  
TCGGATCTCAAGTACCCACTGATCCATGGACAGGGTAGGCAGGCTCGG  
TTACTAGGAACACTGGCTGTCTCCCGGGGCCTGGGAGACCATCAGCTC  
AGAGTCCTGGACACAAACATCCAGCTCAAGCCCTTCTTGCTCTCTGTG  
CCACAGGTGACTGTGCTGGATGTGGACCAGCTGGAGCTACAGGAGGAT  
GATGTGGTTGTTCATGGCAACTGATGGACTCTGGGATGTACTGTCCAAC  
GAGCAGGTGGCATGGCTGGTGCAGGAGCTTCCTCCCTGGGAACCAAGAG  
GACCCACACAGGTTCCTCAAAGCTGGCCAGATGCTGATACACAGCACA  
CAGGGAAAGGAAGACAGTCTCACAGAGGAAGGGCAGGTGCCTACGAT  
GACGTCTCTGTGTTTCGTGATTCCCTTGCACAGTCAGGGCCAAGAGAGC  
AGTGACCACTgagcggccgc

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