

*Division of Signal Transduction Therapy*

**Standard Operating Procedure**

**Preparation of PPM1H D288E [1 – 514]**

**Enzyme description:-** PPM1H D288E [1 – 514]

**Clone number:-** DU 68088

**Source:-** Recombinant

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

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

**Purification method:-** Ni<sup>2+</sup>-NTA agarose

**Calculated molecular mass:-**

Monoisotopic          68, 413.51 daltons  
Average Mass          68, 456.46 daltons  
[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 6.08

**Purity:-** >80 %

**Enzyme storage buffer:-**

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

**Storage temperature:-** -70 °C

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## Clone Data Sheet

### PPM1H D288E [1 - 514]

**Protein** PPM1H D288E [1 - 514]

**Clone number** DU 68088

**Species** Human

**Accession number** Q9ULR3.2

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

**Bacterially expressed PPM1H protein** MGHHHHHS DQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMT  
THLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIE  
VYQEQTGGMLTRVKSAVANFMGGIMAGSSGSEHGGGSCGGSDLPLRFP  
YGRPEFLGLSQDEVECSADHIARPILILKETRRLPWATGYAEVINAGK  
STHNEDQASCEVLTVKKKAGAVTSTPNRNSSKRRSSLPNGEGLQLKEN  
SESEGVSCHYWSLFDGHAGSGAAVVASRLLQHHITEQLQDIVDILKNS  
AVLPPTCLGEEPENTPANSRTLTRAASLRGGVGAPGSPSTPPTRFFTE  
KKIPHECLVIGALESFAFKEMDLQIERERSSYNISGGCTALIVICLLGK  
LYVANAGE SRAIIIRNGEIIIPMSSEFTPETERQRLQYLAFMQPHLLGN  
EFTHLEFP RRVRKELGKKMLYRDFNMTGWAYKTIEDEDLKFPLIYGE  
GKKARVMATIGVTRGLGDHDLKVHDSNIYIKPFLSSAPEVRIYDLSKY  
DHGSDDVLILATDGLWDVLSNEEVAEAITQFLPNCDDPHRYTLAAQ  
DLVMRARGVLKDRGWRI SNDR LGS GDDISVYVIPLIHGNKLS

**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 D288E mutation. Residue D288 is equivalent to E392 of the fusion protein.

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

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Cloning sites

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

Complete  
Nucleotide  
Sequence

ATGCTCACTCGAGTGAAATCTGCCGTGGCCAATTTTCATGGGCGGCATC  
ATGGCTGGCAGCTCAGGCTCCGAGCACGGCGGGCGGCAGCTGCGGAGGC  
TCGGACCTGCCCCTGCGTTTCCCCTACGGGCGGCCAGAGTTCTGGGG  
CTGTCTCAGGACGAGGTGGAGTGCAGCGCCGACCACATCGCCCGCCCC  
ATCCTCATCCTCAAGGAGACTCGGCGGCTGCCCTGGGCCACTGGCTAC  
GCAGAGGTTATCAATGCCGGGAAGAGCACACACAATGAAGACCAAGCC  
AGCTGTGAGGTGCTCACTGTGAAGAAGAAGGCAGGGGCGGTGACCTCA  
ACCCCAAATAGGAACTCATCCAAGAGACGGTCTCCCTTCCCAATGGG  
GAAGGGCTGCAGCTGAAGGAGAACTCGGAATCCGAGGGTGTTCCTGC  
CACTATTGGTCGCTGTTTGACGGGCACGCGGGGTCCGGGGCCGCGGTG  
GTGGCGTCACGCCTGCTGCAGCACCACATCACGGAGCAGCTGCAGGAC  
ATCGTGGACATCCTGAAGAACTCCGCCGTCTGCCCCCTACCTGCCTG  
GGGAGGAGCCTGAGAACACGCCCGCCAACAGCCGGACTCTGACCCGG  
GCAGCCTCCCTGCGCGGAGGGGTGGGGCCCCGGGCTCCCCCAGCACG  
CCCCCACACGCTTCTTTACCGAGAAGAAGATTCCCCATGAGTGCCTG  
GTCATCGGAGCGCTTGAAAGTGCATTCAAGGAAATGGACCTACAGATA  
GAACGAGAGAGGAGTTCATATAATATATCTGGTGGCTGCACGGCCCTC  
ATTGTGATTTGCCTTTTGGGGAAGCTGTATGTTGCAAATGCTGGGGAA  
AGCAGGGCCATAATCATCAGAAATGGAGAAATTATCCCCATGTCTTCA  
GAATTTACCCCCGAGACGGAGCGCCAGCGACTTCAGTACCTGGCATTTC  
ATGCAGCCTCACTTGCTGGGAAATGAGTTCACACATTTGGAGTTTCCA  
AGGAGAGTACAGAGAAAGGAGCTTGGAAAGAAGATGCTCTACAGGGAC  
TTTAATATGACAGGCTGGGCATACAAAACCATTGAGGATGAGGACTTG  
AAGTTCCCCCTTATATATGGAGAAGGCAAGAAGGCCCGGGTAATGGCA  
ACTATTGGAGTGACCAGGGGACTTGGGGACCATGACCTGAAGGTGCAT  
GACTCCAACATCTACATTAACCATTTCCTGTCTTCAGCTCCAGAGGTA  
AGAATCTACGATCTTTCAAATATGATCATGGATCAGATGATGTGCTG  
ATCTTGGCCACTGATGGACTCTGGGACGTTTTATCAAATGAAGAAGTA  
GCAGAAGCAATCACTCAGTTTCTTCCTAACTGTGATCCAGATGATCCT  
CACAGGTACACACTGGCAGCTCAGGACCTGGTGTGCGTGCCGGGGT  
GTGCTGAAGGACAGAGGATGGCGGATATCTAATGACCGACTGGGCTCA  
GGAGACGACATTTCTGTATATGTCATTCCTTTAATACATGGAAACAAG  
CTGTCAtgagcggccgcg

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