MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of PPM1J [1 – 505]

Enzyme description:- PPM1J [1 – 505]

Clone number:- DU 68140

Source:- Recombinant

Expression system:- *E.coli*,

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

Purification method:- Cobalt agarose

Calculated molecular mass:-

Monoisotopic	66, 786.66 daltons
Average Mass	66, 828.70 daltons

[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.53

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

PPM1J [1 - 505]

Protein	PPM1J [1 - 505]
Clone number	DU 68140
Species	Human
Accession number	NM_005167.5
Tags	N-terminal His(6) + SUMO
Bacterially expressed PPM1J protein	MGHHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMT THLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIE VYQEQTGG**MLNRVRSAVAHLVSSGGAPPPRPKSPDLPNAASAPPAAAP** **EAPRSPPAKAGSGSATPAKAVEARASFSRPTFLQLSPGGLRRADDHAG** **RAVQSPPDTGRRLPWSTGYAEVINAGKSRHNEDQACCEVVYVEGRRSV** **TGVPREPSRGQGLCFYYWGLFDGHAGGGAAEMASRLLHRHIREQLKDL** **VEILQDPSPPPLCLPTTPGTPDSSDPSHLLGPQSCWSSQKEVSHESLV** **VGAVENAFQLMDEQMARERRGHQVEGGCCALVVIYLLGKVYVANAGDS** **RAIIVRNGEIIPMSREFTPETERQRLQLLGFLKPELLGSEFTHLEFPR** **RVLPKELGQRMLYRDQNMTGWAYKKIELEDLRFPLVCGEGKKARVMAT** **IGVTRGLGDHSLKVCSSTLPIKPFLSCFPEVRVYDLTQYEHCPDDVLV** **LGTDGLWDVTTDCEVAATVDRVLSAYEPNDHSRYTALAQALVLGARGT** **PRDRGWRLPNNKLGSGDDISVFVIPLGGPGSYS**
Native sequence	Amino acids M1 – S505 (end) of human PPM1J. 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

Complete Nucleotide Sequence

ATGGGTCATCATCACCATCACCATTCTGACCAGGAGGCAAAACCTTCA
ACTGAGGACTTGGGGGATAAGAAGGAAGGTGAATATATTAAACTCAAA
GTCATTGGACAGGATAGCAGTGAGATTCACTTCAAAGTGAAAATGACA
ACACATCTCAAGAAACTCAAAGAATCATACTGTCAAAGACAGGGTGTT
CCAATGAACTCACTCAGGTTTCTCTTTGAGGGTCAGAGAATTGCTGAT
AATCATACTCCAAAAGAACTGGGAATGGAGGAAGAAGATGTGATTGAA
GTTTATCAGGAACAAACGGGGGGAATGCTAAACCGGGTGCGCTCGGCC
GTGGCGCACCTGGTGAGCTCCGGGGGCGCTCCGCCTCCGCGCCCCAAA
TCCCCGGACCTGCCCAACGCCGCCTCGGCGCCGCCCGCCGCCGCTCCA
GAAGCGCCCAGGAGCCCTCCCGCGAAGGCTGGGAGCGGGAGCGCGACG
CCCGCGAAGGCTGTTGAGGCTCGAGCGAGCTTCTCCAGACCGACCTTT
CTGCAGCTGAGCCCCGGGGGGCTGCGACGCGCCGATGACCACGCGGGC
CGGGCTGTGCAAAGCCCCCCGGACACGGGCCGCCGCCTGCCCTGGAGC
ACAGGCTACGCCGAGGTCATCAATGCTGGCAAGAGTCGGCACAATGAG
GACCAGGCTTGCTGTGAAGTGGTGTATGTGGAAGGTCGGAGGAGTGTT
ACAGGAGTACCTAGGGAGCCTAGCCGAGGCCAGGGACTCTGCTTCTAC
TACTGGGGCCTATTTGATGGGCATGCAGGGGGCGGAGCTGCTGAAATG
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GTAGAGATACTTCAGGACCCTTCGCCACCACCCCTCTGCCTCCCAACC
ACTCCGGGGACCCCAGATTCCTCCGATCCCTCTCACTTGCTTGGCCCT
CAGTCCTGCTGGTCTTCACAGAAGGAAGTGAGCCACGAGAGCCTGGTA
GTGGGGGCCGTTGAGAATGCCTTCCAGCTCATGGATGAGCAGATGGCC
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TTTCCTCTGGTCTGTGGGGAGGGCAAAAAGGCTCGGGTGATGGCCACC
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TCCACCCTGCCCATCAAGCCCTTTCTCTCCTGCTTCCCTGAGGTACGA
GTGTATGACCTGACACAATATGAGCACTGCCCAGATGATGTGCTAGTC
CTGGGAACAGATGGCCTGTGGGATGTCACTACTGACTGTGAGGTAGCT
GCCACTGTGGACAGGGTGCTGTCGGCCTATGAGCCTAATGACCACAGC
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CCCCGAGACCGTGGCTGGCGTCTCCCCAACAACAAGCTGGGTTCCGGG
GATGACATCTCTGTCTTCGTCATCCCCCTGGGAGGGCCAGGCAGTTAC
TCCtgagcggccgc