

MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of Lambda Phosphatase [3 - 221]

Enzyme description:- Lambda Protein Phosphatase [3 – 221]

Clone number:- DU 4170

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GSTand C-Terminal His6

Purification method:- GSH Sepharose.

Calculated molecular mass:-

Monoisotopic 52, 544.52 daltons

Average Mass 52, 578.40 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.82

Purity:- >80 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

Lambda Protein Phosphatase [3 – 221]

<u>Protein</u>	Lambda Phosphatase [3 – 221]
<u>Clone number</u>	DU 4170
<u>Species</u>	Bacteriophage lambda
<u>Accession number</u>	AAA96594.1
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAETSMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFGQPLGSYYEKIDGSKYRNIW VVGDLHGCTNLMNKLDTIGFDNKKDLLISVGDLVDRGAENVECLELIT FPWFR AVRGNHEQMMIDGLSERGNVNHWLLNGGGWFFNLDYDKEILAKA LAHKADELPLIIELVSKDKKYVICHADYPFDEYEFGKPV DHQQVIWNRE RISNSQNGIVKEIKGADTFIFGHTPAVKPLKFANQMYIDTGAVFCGNLT LIQVQGEGAHHHHHH
<u>Native sequence</u>	Amino acids Y3 – A221 (end) of Bacteriophage Lambda Phosphatase.. Residue Y232 of the fusion protein is equivalent to Y3 of the native enzyme. The GST tag is located at residues 1 – 220 and the His6 tag is located at residues 451 - 456.
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> I sites of pGEX6P-1

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Complete Nucleotide Sequence

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCA
CTCGACTTCTTTTGAATATCTTGAAGAAAAATATGAAGAGCATTTGTA
TGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGT
TTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAA
CACAGTCTATGGCCATCATACTGTTATATAGCTGACAAGCACAAACATGTT
GGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCG
GTTTTGGATATTAGATACGGTGTTCGAGAATTGCATATAGTAAAGACT
TTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAA
AATGTTCTGAAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCAT
GTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTAT
ACATGGACCCAATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAA
AAAACGTATTGAAGCTATCCACAAATTGATAAGTACTTGAAATCCAGC
AAGTATATAGCATGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTG
GCGACCATCCTCCAAAATCGGATCTGGAAGTTCTGTTCCAGGGGCCCT
GGGATCCTATTACGAAAAAATTGATGGCAGCAAATACCGAAATATTTGG
GTAGTTGGCGATCTGCACGGATGCTACACGAACCTGATGAACAACTGG
ATACGATTGGATTTCGACAACAAAAAAGACCTGCTTATCTCGGTGGGCGA
TTTGGTTGATCGTGGTGCAGAGAACGTTGAATGCCTGGAATTAATCACA
TTCCCCTGGTTCAGAGCTGTACGTGGAAACCATGAGCAAATGATGATTG
ATGGCTTATCAGAGCGTGGAAACGTTAATCACTGGCTGCTTAATGGCGG
TGGCTGGTTCTTTAATCTCGATTACGACAAAGAAATTCTGGCTAAAGCT
CTTGCCCATAAAGCAGATGAACTTCCGTTAATCATCGAACTGGTGAGCA
AAGATAAAAAATATGTTATCTGCCACGCCGATTATCCCTTTGACGAATA
CGAGTTTGGAAAGCCAGTTGATCATCAGCAGGTAATCTGGAACCGCGAA
CGAATCAGCAACTCACAAAACGGGATCGTGAAAGAAATCAAAGGCGCGG
ACACGTTTCATCTTTGGTCATACGCCAGCAGTGAAACCACTCAAGTTTGC
CAACCAAATGTATATCGATACCGGCGCAGTGTTCTGCGGAAACCTAACA
TTGATTTCAGGTACAGGGAGAAGGCGCACACCATCACCATt_{gag}
cggccgc