

Certificate of Analysis

LMPTP-A, active

(Recombinant enzyme expressed in *E. coli* cells)

Item # 14-619, 14-619-K, 14-619M

Parent Lot # 1654886

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal GST-tagged, recombinant, human LMPTP-A, full length, expressed in *E. coli* cells. Purified using glutathione agarose. Purity 94.1% by SDS-PAGE and Coomassie blue staining. MW = 44.5kDa.

Specific Activity (Parent lot# 1654886): 8243U/mg, where one unit of LMPTP-A activity is defined as the release of 1nmol of phosphate per minute from the phosphorylated substrate 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP) at room temperature.

Formulation: 2.075mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 10% glycerol, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

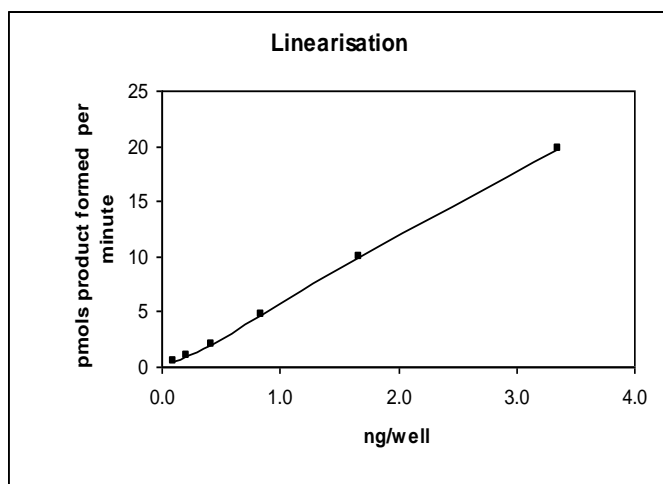
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

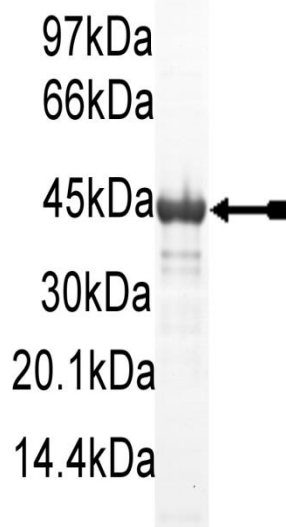
Quality Control Testing

Phosphatase Assay: 0.1–3.4ng of this lot of enzyme dephosphorylated 200µM DiFMUP in the assay described on page two. Assay background was subtracted from the actual Fluorescence Intensity (FI) to yield the results shown below. Quantification of FI was against a 6,8-difluoro-7-hydroxy-4-methylcoumarin (DiFMU) standard curve.



MS Tryptic Fingerprint: Confirmed product identity as LMPTP-A with the translated sequence listed on page three.

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of LMPTP-A, active.



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Phosphatase Assay Protocol

Stock Solutions:

1. **Reaction Buffer:** 32mM Na-Acetate pH4.5, 50mM NaCl, 2.5mM EDTA, 0.17mM DTT, 0.83(v/v)% glycerol, 0.017(w/v)% BSA, 0.002% Brij-35
2. 500µM DiFMUP (Molecular Probes Catalogue# D6567) in water.
3. 100mM sodium orthovanadate.
4. 500µM DiFMU (Molecular Probes Catalogue # D6566) in water for the calibration curve.

Assay Procedure:

1. Dilute **LMPTP-A, active** in reaction buffer and use **0.1–3.4ng** in 15µl per assay point.
2. Add 10µl DiFMUP 500µM stock solution (200µM final assay concentration.)
3. Incubate for 30 minutes at room temperature.
4. Stop the reaction by adding 5µl of 100mM sodium orthovanadate.
5. Read FI using an appropriate reader (Excitation 340nm; Emission 450nm.)
6. Subtract the zero enzyme values from each FI reading and calculate the enzyme activity by conversion to nmoles product formed using a DiFMU standard calibration curve.

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LMPTP-A Sequence Information

| | |
|--------------------------------|--|
| <u>Protein</u> | Human LMPTP-A |
| <u>Tags</u> | N-terminal GST |
| <u>Native sequence</u> | M227 of the recombinant protein is equivalent to M1 of human LMPTP-A |
| <u>Accession number</u> | GenBank NM_004300. The recombinant protein contains the amino acid substitution Q106R (native coordinates) with reference to GenBank NM_004300. This conflict is reported in GenBank accession numbers S62884 and M87545. |

Recombinant LMPTP-A amino acid sequence:

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1  MSPILGYWKI  KGLVQPTRL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQ SMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121  DFLSKLPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181  KRIEAI PQID  KYLKSSKYIA  WPLQGQWATF  GGGDHPPKSD  LVPRGSM AEQ  ATKSVLFVCL
241  GNICRSPIAE  AVFRKLVTDQ  NISENWRVDS  AATSGYEIGN  PPDYRGQSCM  KRHGIPMSHV
301  ARQITKEDFA  TFDYILCMDE  SNLRDLNRKS  NRVKTCKAKI  ELLGSYDPQK  QLIIEDPYYG
361  NDSDFETVYQ  QCVRCCRAFL  EKAH

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Recombinant LMPTP-A nucleotide sequence:

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1  atgtccccta  tactaggtta  ttgaaaatt  aagggccttg  tgcaaccac  tgcacttctt
61  ttggaatata  ttgaagaaaa  atatgaagag  catttgtatg  agcgcgatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggttgg  gagtttccca  atcttcctta  ttatatgtat
181  ggtgatgtta  aattaacaca  gtctatggcc  atcatacgtt  atatagctga  caagcacaac
241  atgttgggtg  gttgtccaaa  agagcgtgca  gagatttcaa  tgcttgaagg  agcggttttg
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361  gattttctta  gcaagctacc  tgaaatgctg  aaaatgttct  aagatcgttt  atgtcataaa
421  acatatttaa  atggtgatca  tgtaaccat  cctgacttca  tgttgtatga  cgctcttgat
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1141  gagaaggccc  actga

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