

## Certificate of Analysis

### LMPTP-B, active

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

Item # 14-620, 14-620-K, 14-620M

Parent Lot # LB226P60

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

**Product Description:** N-terminal GST-tagged, recombinant, human LMPTP-B, full length, expressed in *E. coli* cells. Purified using glutathione agarose. Purity 100% by SDS-PAGE and Coomassie blue staining. MW = 44kDa.

**Specific Activity (Parent lot# LB226P60):** 13500U/mg, where one unit of LMPTP-B 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:** 3.777mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

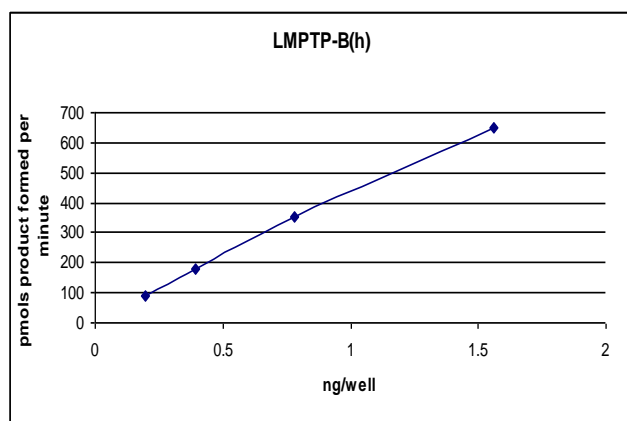
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

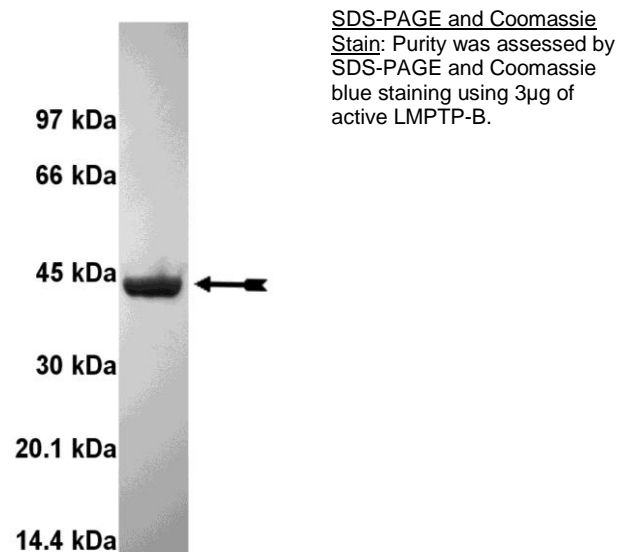
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Phosphatase Assay:** 0.2–1.5ng 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 identity as LMPTP-B with the translated sequence listed on page three.



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### Phosphatase Assay Protocol

#### Stock Solutions:

1. **Reaction Buffer:** 60mM Hepes pH7.2, 150mM NaCl, 1mM EDTA, 0.17mM DTT, 0.83 (v/v)% glycerol, 0.017 (w/v)% BSA, 0.002% Brij-35.
2. 500 $\mu$ M DiFMUP (Molecular Probes Catalogue# D6567) in water.
3. 100mM sodium orthovanadate.
4. 500 $\mu$ M DiFMU (Molecular Probes Catalogue# D6566) in water for the calibration curve.

#### Assay Procedure:

1. Dilute **LMPTP-B** in reaction buffer and use 0.2–1.5ng in 15 $\mu$ l per assay point.
2. Add 10 $\mu$ l DiFMUP 500 $\mu$ M stock solution (200 $\mu$ M final assay concentration).
3. Incubate for 30 minutes at room temperature.
4. Stop the reaction by adding 5 $\mu$ l 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-B Sequence Information

<b><u>Protein</u></b>	Human LMPTP-B
<b><u>Tags</u></b>	N-terminal GST
<b><u>Native sequence</u></b>	M227 of the recombinant protein is equivalent to M1 of human LMPTP-B
<b><u>Accession number</u></b>	GenBank NM_007099

#### **Recombinant LMPTP-B amino acid sequence:**

```

1  MSPIILGYWKI  KGLVQPTRL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121  DFLSKLP EML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181  KRIEAIPQID  KYLKSSKYIA  WPLQGQWATF  GGGDHPPKSD  LVPRGSMAEQ  ATKSVLFCVCL
241  GNICRSPIAE  AVFRKLVTDQ  NISENWVIDS  GAVSDWNVGR  SPDPRVAVSCL  RNHGIHTAHK
301  ARQITKEDFA  TFDYILCMDE  SNLRDLNRKS  NQVKTCKAKI  ELLGSYDPQK  QLIIEDPYYG
361  NDSDFETVYQ  QCVRCCRAFL  EKAH
  
```

#### **Recombinant LMPTP-B nucleotide sequence:**

```

1  atgtccccta  tactaggtta  ttgaaaatt  aagggccttg  tgcaaccac  tgcacttctt
61  ttggaatata  ttgaagaaaa  atatgaagag  catttgatg  agcgcgatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatattgat
181  ggtgatgtta  aattaacaca  gtctatggcc  atcatacggt  atatagctga  caagcacaac
241  atgttgggtg  gttgtccaaa  agagcgtgca  gagatttcaa  tgcttgaagg  agcggttttg
301  gatattagat  acgggtgttc  gagaattgca  tatagtaaag  actttgaaac  tctcaaagtt
361  gattttctta  gcaagctacc  tgaaatgctg  aaaatgttcg  aagatcgttt  atgtcataaa
421  acatatttaa  atggatgatca  tgtaaccat  cctgacttca  tgttgatga  cgctcttgat
481  gttgttttat  acatggacc  aatgtgcctg  gatgcgttcc  caaaattagt  ttgttttaaa
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901  gcaagacaga  ttaccaaaga  agattttgcc  acatttgatt  atatactatg  tatggatgaa
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1021  gaactacttg  ggagctatga  tccacaaaa  caacttatta  ttgaagatcc  ctattatggg
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1141  gagaaggccc  actga
  
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