

## Certificate of Analysis

### PTP-1B, active

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

Item # 14-621, 14-621-K, 14-621M

Parent Lot # D8EN018U

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 PTP-1B, amino acids 1–321 expressed in *E. coli* cells. Purified using glutathione- agarose. Purity 46% by SDS-PAGE and Coomassie blue staining. MW = 64.1kDa.

**Specific Activity (Parent lot# D8EN018U):** 11537U/mg, where one unit of PTP-1B, active 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:** 0.637mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 10% glycerol, 0.1mM EGTA, 0.03% Brij-35, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

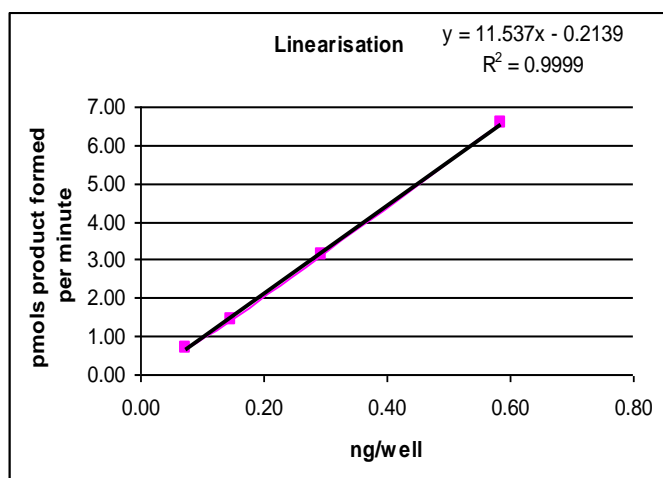
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

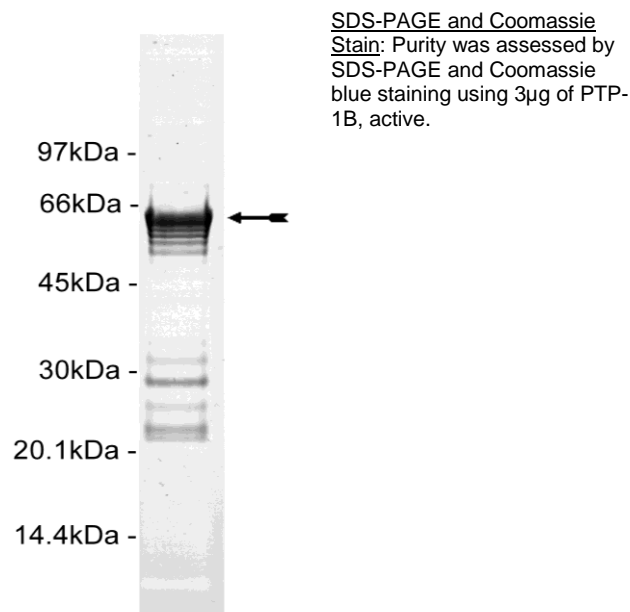
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Phosphatase Assay:** 0.07–0.59ng 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 PTP-1B with the translated sequence listed on page three.



## Certificate of Analysis

### Phosphatase Assay Protocol

#### Stock Solutions:

1. **Reaction Buffer:** 32mM Hepes pH7.2, 50mM NaCl, 2.5mM 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 **PTP-1B** in reaction buffer and use 0.07–0.59ng 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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### PTP-1B Sequence Information

<b><u>Protein</u></b>	Human PTP-1B (1–321)
<b><u>Tags</u></b>	N-Terminal GST
<b><u>Native sequence</u></b>	M230 of the recombinant protein is equivalent to M1 of human PTP-1B
<b><u>Accession number</u></b>	GenBank NM_002827

#### **Recombinant PTP-1B amino acid sequence:**

```

1  MSPILGYWKI  KGLVQPTRLL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQ SMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121  DFLSKLP EML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181  KRIEAIPQID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LVPRGSPEFM  EMEKEFEQID
241  KSGSWAAIYQ  DIRHEADSFP  CRVAKLPKKN  NRRNRYRDVSP  FDHSRIKLNH  EDNDYINASL
301  IKMEEAQRSY  ILTQGPLPNT  CGHFWEMVWE  QKSRGVVMLN  RVMEKGSCLK  AQYWPQKEEK
361  EMIFEDTNLK  LTLISEDIKS  YYTVRQLELE  NLTTQETREI  LHFHYTTWPD  FGVPEPSPAS  F
421  LNFLFKVRES  GSLSPEHGVP  VVHCSAGIGR  SGTFCCLADTC  LLLMDKRKDP  SSVDIKKVLL
481  EMRKFRMGLI  QTADQLRFSY  LAVIEGAKFI  MGDSSVQDQW  KELSHEDLEP  PPEHI PPPPR
541  PPKRILEPHN

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#### **Recombinant PTP-1B nucleotide sequence:**

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1  atgtccccta  tactaggtta  ttgaaaatt  aaggccttg  tgcaaccac  tcgacttctt
61  ttggaatata  ttgaagaaaa  atatgaagag  catttgatg  agcgcgatga  aggtgataaa
121  tggcgaaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatattgat
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1621  ccacccaac  gaatcctgga  gccacacaat  tag

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