

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.

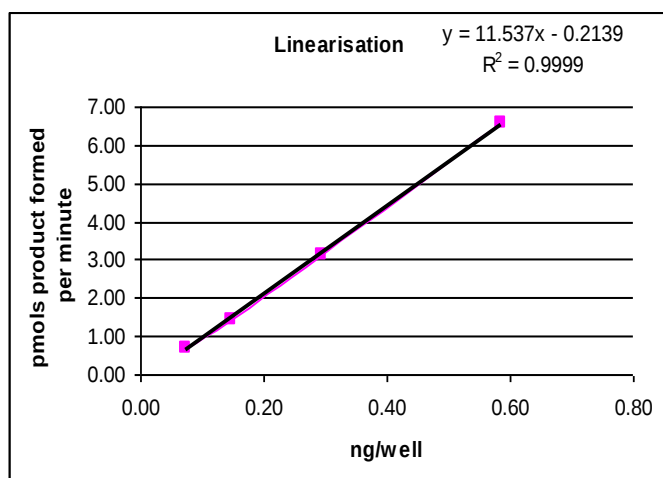
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

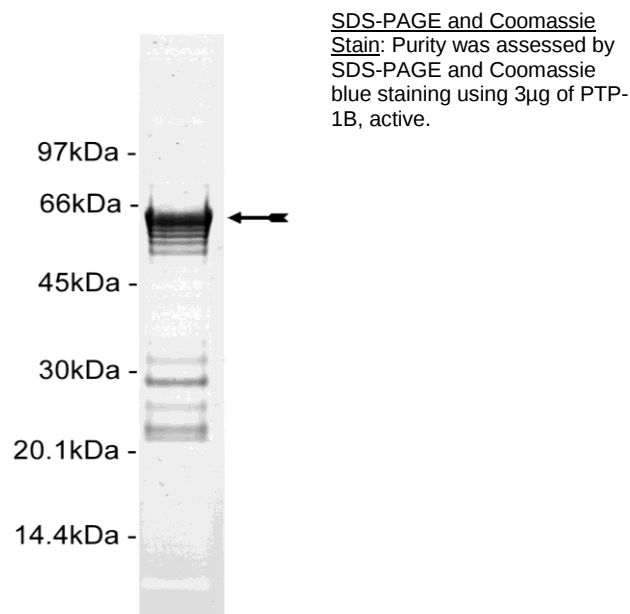
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

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. 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 **PTP-1B** in reaction buffer and use 0.07–0.59ng 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.

Certificate of Analysis

PTP-1B Sequence Information

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

Recombinant PTP-1B amino acid sequence:

```

1  MSPILGYWKI  KGLVQPTRLL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121 DFLSKLPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181 KRIEAIQID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LVPRGSPEFM  EMEKEFEQID
241 KSGSWAAIYQ  DIRHEADFP  CRVAKLPKNK  NNRNRYRDVSP  FDHSRIKLHQ  EDNDYINASL
301 IKMEEAQRSY  ILTQGPLPNT  CGHFWEMVWE  QKSRGVVMLN  RVMEKGSCLK  AQYWPQKEEK
361 EMIFEDTNLK  LTLISEDIKS  YYTVRQLELE  NLTTQETREI  LHFHYTTWPD  FGVPEPASPF
421 LNFLFKVRES  GSLSPHEGPV  VVHCSAGIGR  SGTFCCLADTC  LLLMDKRKDP  SSVDIKKVLL
481 EMRKFRMGDI  QTADQLRFSY  LAVIEGAKFI  MGDSSVQDQW  KELSHEDLEP  PPEHI PPPPR
541 PPKRILEPHN

```

Recombinant PTP-1B nucleotide sequence:

```

1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tgcacttctt
61  ttggaatata  ttgaagaaaa  atatgaagag  catttgatat  agcgcgatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatatattg
181  ggtgatgtta  aattaacaca  gtctatggcc  atcatagctt  atataagctg  caagcacaa
241  atgttgggtg  gttgtccaaa  agagcgtgca  gagatttcaa  tgcttgaagg  agcggttttg
301  gatattagat  acggtgtttc  gagaattgca  tatagtaaa  actttgaaac  tctcaaagtt
361  gattttctta  gcaagctacc  tgaaatgctg  aaaatgttcg  aagatcgttt  atgtcataaa
421  acataattta  atgggtgatc  tgtaacccat  cctgacttca  tggttgatga  cgctcttgat
481  gttgttttat  acatggaccc  aatgtgcctg  gatgcgttcc  caaaattagt  ttgtttttaa
541  aaacgtattg  aagctatccc  acaaattgat  aagtacttga  aatccagcaa  gtatatagca
601  tggcctttgc  agggctggca  agccacgttt  ggtggtggcg  accatcctcc  aaaatcggat
661  ctggttccgc  gtggatcccc  ggaattcatg  gagatggaaa  aggagtccga  gcagatcgac
721  aagtccggga  gctgggcggc  catttaccag  gatatccgac  atgaagccag  tgacttccca
781  tgtagagtgg  ccaagcttcc  taagaacaaa  aaccgaaata  ggtacagaga  cgtcagtcce
841  tttgaccata  gtcggattaa  actacatcaa  gaagataatg  actatatcaa  cgctagtttg
901  ataaaaatgg  aagaagccca  aaggagttac  attcttacc  agggcccttt  gcctaacaca
961  tgcggtcact  tttgggagat  ggttgaggag  cagaaaagca  ggggtgtcgt  catgctcaac
1021 agagtgatgg  agaaagggtc  gttaaaatgc  gcacaatact  ggccacaaaa  agaagaaaaa
1081 gagatgatct  ttgaagacac  aaatttgaaa  ttaacattga  tctctgaaga  tatcaagtca
1141 tattatacac  tgcgacagct  agaattggaa  aaccttaca  cccaagaaac  tcgagagatc
1201 ttacatttcc  actataccac  atggcctgac  tttggagtcc  ctgaatcacc  agcctcattc
1261 ttgaactttc  ttttcaaagt  ccgagagtca  gggctactca  gcccgagaca  cgggcccgtt
1321 gtggtgcact  gcagtgcagg  catcggcagg  tctggaacct  tctgtctggc  tgatacctgc
1381 ctcttgctga  tggacaagag  gaaagacctt  tcttccgttg  atatcaagaa  agtgctgtta
1441 gaaatgagga  agtttcggat  ggggtgatc  cagacagccg  accagctgag  cttctcctac
1501 ctggctgtga  tcgaaggtgc  caaattcatc  atgggggact  cttccgtgca  ggatcagtgg
1561 aaggagcttt  cccacgagga  cctggagccc  ccaccgagc  atatcccccc  acctcccccg
1621 ccacccaaac  gaatcctgga  gccacacaat  tag

```

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

© 2014 Eurofins Pharma Discovery Services UK Limited is an independent member of Eurofins Discovery Services.