

Certificate of Analysis

HePTP, active

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

Item # 14-593, 14-593-K, 14-593M

Parent Lot # 1922202

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 6His-tagged, recombinant, human HePTP, amino acids 22–end, expressed in *E. coli* cells. Purified using Ni^{2+} /NTA agarose. Purity 72.9% by SDS-PAGE and Coomassie blue staining. MW = 40.7kDa.

Specific Activity (Parent lot# 1922202): 8316U/mg, where one unit of HePTP, 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: 0.812mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 10% glycerol, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

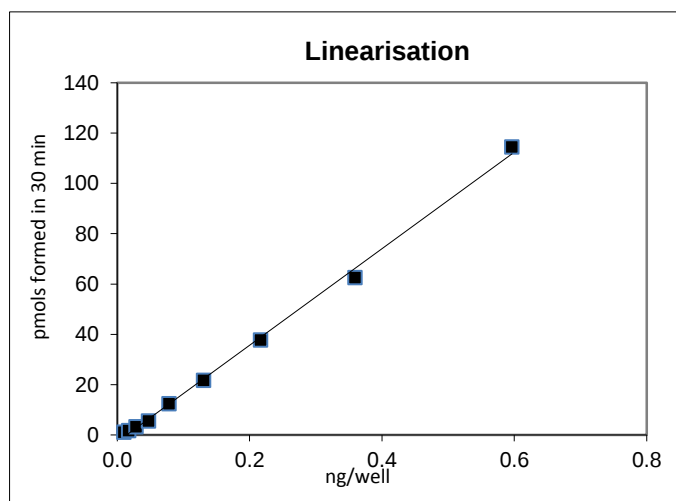
Storage and Stability: On receipt of material store at -70°C . Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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 .

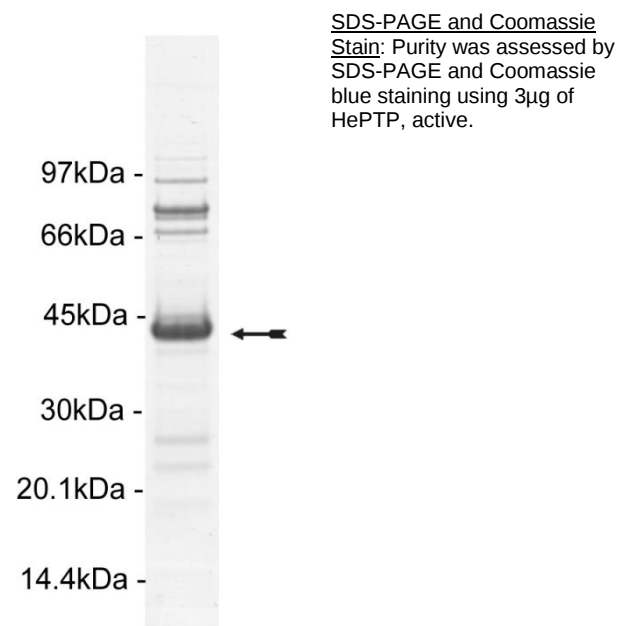
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Phosphatase Assay: 0.01–0.6ng of this lot of enzyme dephosphorylated 200 μM 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 HePTP 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. 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 **HePTP** in reaction buffer and use 0.01–0.6ng 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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HePTP Sequence Information

<u>Protein</u>	HePTP
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M21 of the recombinant protein is equivalent to M22 of human HePTP
<u>Accession number</u>	GenBank NM_002832

Recombinant HePTP amino acid sequence:

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1  MGSSHHHHHH  SSGLVPRGSH  MTQPPPEKTP  AKKHVRLQER  RGSNVALMLD  VRSLGAVEPI
61  CSVNTPREVT  LHFLRTAGHP  LTRWALQRQP  PSPKQLEEEF  LKIPSNFVSP  EDLDIPGHAS
121  KDRYKTILPN  PQSRVCLGRA  QSQEDGDYIN  ANYIRGYDGK  EKVIYATQGP  MPNTVSDFWF
181  MVWQEEVSLI  VMLTQLREGK  EKCVDHYWPE  EETYGPFQIR  IQDMKECPEY  TVRQLTIQYQ
241  EERRSVKHIL  FSAWPDHQT  ESAGPLLRLV  AEVEESPETA  AHPGPIVVHC  SAGIGRTGCF
301  IATRIGCQQL  KARGEVDILG  IVCQLRLDRG  GMIQTAEQYQ  FLHHTLALYA  GQLPEEPS

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Recombinant HePTP nucleotide sequence:

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1  atgggcagca  gccatcatca  tcatcatcac  agcagcggcc  tgggtgccgcg  cggcagccat
61  atgaccagc  ctccgcctga  aaaaacgcca  gccagaagc  atgtgcgact  gcaggagagg
121  cggggctcca  atgtggtct  gatgctggac  gttcgggtcc  tgggggcccgt  agaaccatc
181  tgctctgtga  acacacccc  ggaggtcacc  ctacactttc  tgcgcactgc  tggacacccc
241  cttaccgct  gggcccttca  gcgcagacca  cccagcccca  agcaactgga  agaagaattc
301  ttgaagatcc  cttcaaactt  tgtcagcccc  gaagacctgg  acatccctgg  ccacgcctcc
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