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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.

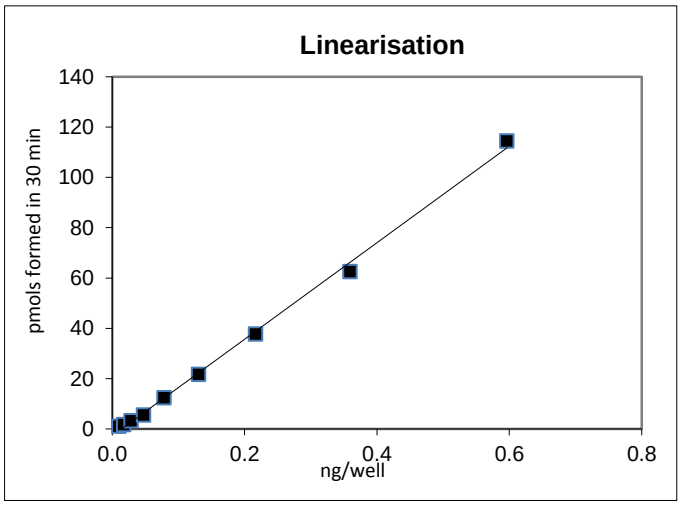
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

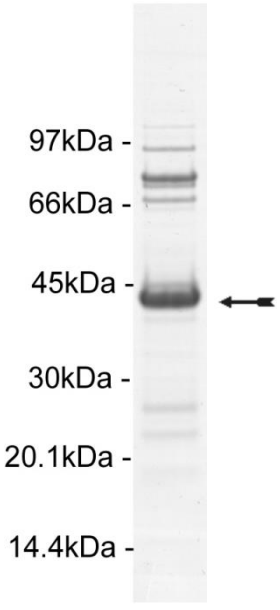
Quality Control Testing

Phosphatase Assay: 0.01–0.6ng of this lot of enzyme dephosphorylated 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 HePTP 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 HePTP, active.



Eurofins Pharma Discovery Services UK Limited
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom
T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

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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

Protein HePTP

Tags *N*-terminal 6His

Native sequence M21 of the recombinant protein is equivalent to M22 of human HePTP

Accession number 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 EKVYIATQGP MPNTVSDFWE
181 MVWQEEVSLI VMLTQLREGK EKCVHYWPTE EETYGPFQIR IQDMKECPEY TVRQLTIQYQ
241 EERRSVKHIL FSAWPDHQTP ESAGPLLRLV AEVEESPETA AHPGPIVVHC SAGIGRTGCF
301 IATRIGCQQL KARGEVDILG IVCQLRLDRG GMIQTAEQYQ FLHHTLALYA GQLPEEPSP
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Recombinant HePTP nucleotide sequence:

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   1 atgggcagca gccatcatca tcatcatcac agcagcggcc tggtgccgcg cggcagccat
  61 atgacccagc ctccgcctga aaaaacgcca gccaagaagc atgtgcgact gcaggagagg
 121 cggggctcca atgtggctct gatgctggac gttcggtccc tgggggccgt agaacccatc
 181 tgctctgtga acacaccccg ggaggtcacc ctacactttc tgcgcactgc tggacacccc
 241 cttacccgct gggcccttca gcgccagcca cccagcccca agcaactgga agaagaattc
 301 ttgaagatcc cttcaaactt tgtcagcccc gaagacctgg acatccctgg ccacgcctcc
 361 aaggaccgat acaagaccat cttgccaaat ccccagagcc gtgtctgtct aggccgggca
 421 cagagccagg aggacggaga ttacatcaat gccaactaca tccgaggcta tgacgggaag
 481 gagaaggtct acattgccac ccagggcccc atgcccaaca ctgtgtcgga cttctgggag
 541 atggtgtggc aagaggaagt gtccctcatt gtcatgctca ctcagctccg agagggcaag
 601 gagaaatgtg tccactactg gcccacagaa gaggaaacct atggaccctt ccagatccgc
 661 atccaggaca tgaaagagtg cccagaatac actgtgcggc agctcaccat ccagtaccag
 721 gaagagcgcc ggtcagtaaa gcacatcctc ttttcggcct ggccagacca tcagacacca
 781 gaatcagctg ggcccctgct gcgcctagtg gcagaggtgg aggagagccc ggagacagcc
 841 gcccaccccg ggcctatcgt agtccactgc agtgcaggga ttggccggac gggctgcttc
 901 atcgccacgc gaattggctg tcaacagctg aaagcccgag gagaagtgga cattctgggt
 961 attgtgtgcc aactgcggct agacagaggg gggatgatcc agacggcaga gcagtaccag
1021 ttcctgcacc acactttggc cctgtatgca ggccagctgc ctgaggaacc cagcccctga
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Reviewed and approved by site quality representative.