

## Certificate of Analysis

### PP5, active

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

Item # 14-778, 14-778-K, 14-778M

Parent Lot # 1911759

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, full length, human PP5, expressed in *E. coli* cells. Purified using glutathione agarose.

Purity 78.6% by SDS-PAGE and Coomassie blue staining. MW = 83.8kDa.

**Specific Activity (Parent lot# 1911759):**

23U/mg, where one unit of PP5, 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:** 1.453mg/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.

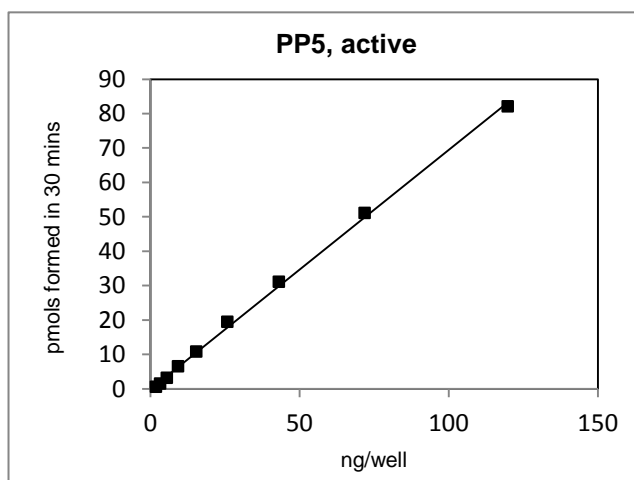
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

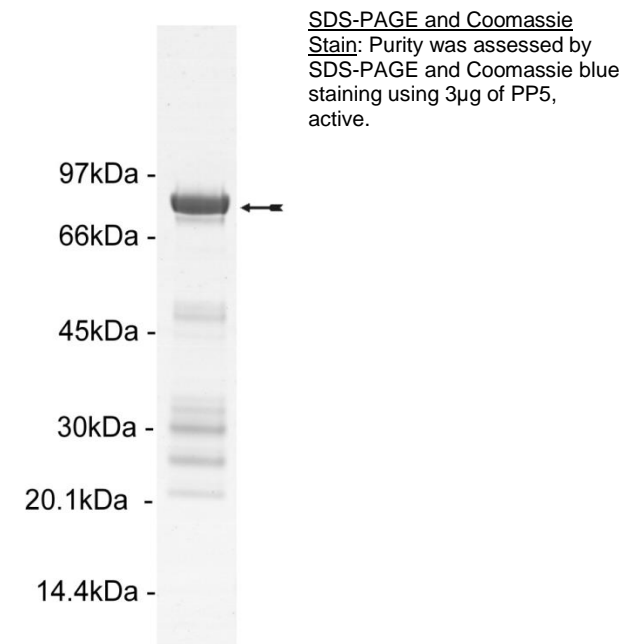
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Phosphatase Assay:** 2.01–119.98ng 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 PP5 with the translated sequence listed on page three



## Certificate of Analysis

### 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 **PP5** in reaction buffer and use 2.01–119.98ng 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.

## Certificate of Analysis

### PP5 Sequence Information

<b><u>Protein</u></b>	Human PP5
<b><u>Tags</u></b>	N-terminal GST
<b><u>Native sequence</u></b>	M232 of the recombinant protein is equivalent to M1 of human PP5
<b><u>Accession number</u></b>	Genbank NM_006247

#### Recombinant PP5 amino acid sequence:

```

1  MSPIILGYWKI  KGLVQPTRL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSM  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121  DFLSKLPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181  KRIEAIPQID  KYLKSSKYIA  WPLQGQWATF  GGGDHPPKSD  LEVLFQGPLG  SMAMAEGERT
241  ECAEPPRDEP  PADGALKRAE  ELKTQANDYF  KAKDYENAIK  FYSQAIELNP  SNAIYYGNRS
301  LAYLRTECYG  YALGDATRAI  ELDKKYIKGY  YRRAASNMAL  GKFRAALRDY  ETVVKVKPHD
361  KDAKMKYQEC  NKIVKQKAFE  RAIAGDEHKR  SVVDSL DIES  MTIEDEYS GP  KLEDGKVTIS
421  FMKELMQWYK  DQKKLHRKCA  YQILVQVKEV  LSKLSTLVET  TLKETEKITV  CGDTHGQFYD
481  LLNIFELNGL  PSETNPYIFN  GDFVDRGSFS  VEVILT LFGF  KLLYPDFHFL  LRGNHETDNM
541  NQIYGFEGEV  KAKYTAQMYE  LFSEVFEWLP  LAQCINGKVL  IMHGGLFSED  GVTLDDIRKI
601  ERNRQPPDSG  PMCDLLWSDP  QPQNGRSISK  RGVSCQFGPD  VTKAFLEENN  LDYIIRSHEV
661  KAEGYEVAHG  GRCVTVFSAP  NYCDQMGNKA  SYIHLQGS DL  RPQFHQFTAV  PHPNVKPMAY
721  ANTL LQLGMM

```

#### Recombinant PP5 nucleotide sequence:

```

1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatata  ttgaagaaaa  atatgaagag  catttgatg  agcgcgatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggttgg  gagtttccca  atcttcctta  ttatattgat
181  ggtgatgtta  aattaacaca  gtctatggcc  atcatacggt  atatagctga  caagcacaac
241  atgttgggtg  gttgtccaaa  agagcgtgca  gagatttcaa  tgcttgaagg  agcggttttg
301  gatattagat  acggtgtttc  gagaattgca  tatagtaaag  actttgaaac  tctcaaagtt
361  gattttctta  gcaagctacc  tgaaatgctg  aaaatgttcg  aagatcgttt  atgtcataaa
421  acatatttaa  atggtgatca  tgtaaccat  cctgacttca  tgttgatga  cgctcttgat
481  gttgttttat  acatggacc  aatgtgctg  gatgcttcc  caaaattagt  ttgttttaa
541  aaacgtattg  aagctatccc  acaaattgat  aagtacttga  aatccagcaa  gtatatagca
601  tggcctttgc  agggctggca  agccacgttt  ggtggtggcg  accatcctcc  aaaatcggat
661  ctggaagttc  tgttccagg  gccctggga  tccatggcga  tggcggagg  cgagaggact
721  gagtgtgctg  agccccccg  ggacgaacc  ccggtgatg  gagctctgaa  gcgggcagag
781  gagctcaaga  ctcaggcaa  tgactacttc  aaagccaagg  actacgagaa  cgccatcaag
841  ttctacagcc  aggccatcga  gctgaacccc  agcaatgcca  tctactatgg  caaccgcagc
901  ctggcctacc  tgcgactga  gtgctatggc  tacgcgctgg  gagacgccac  gcgggccatt
961  gagctggaca  agaagtacat  caagggttat  taccgcccgg  ctgccagcaa  catggcactg
1021  ggcaagttcc  gggccgcgct  gcgagactac  gagacggtgg  tcaaggtgaa  gccccatgac
1081  aaggatgcc  aatgaaata  ccaggagtgc  aacaagatcg  tgaagcagaa  ggcctttgag
1141  cgggccatcg  cgggcgacga  gcacaagcgc  tccgtggtgg  actcgtgga  catcgagagc
1201  atgaccattg  aggatgagta  cagcggacc  aagcttgaag  acggcaaagt  gacaatcagt
1261  ttcatgaagg  agctcatgca  gtggtacaag  gaccagaaga  aactgcaccg  gaaatgtgcc
1321  taccagattc  tggtacaggt  caaagaggtc  ctctccaagc  tgagcacgct  cgtggaaacc
1381  aactcaaag  agacagagaa  gattacagta  tgtggggaca  cccatggcca  gttctatgac
1441  ctctcaaca  tattcgagct  caacggttta  ccctcggaga  ccaaccctta  tatatttaat
1501  ggtgactttg  tggaccgagg  ctcttctct  gtagaagtga  tctcaccct  tttcggcttc
1561  aagctcctgt  accagatca  ctttcacctc  cttcgaggca  accacgagac  agacaacatg

```

## Certificate of Analysis

```
1621 aaccagatct acggtttcga gggtgaggtg aaggccaagt acacagccca gatgtacgag
1681 ctcttttagcg aggtgttcga gtggctcccg ttggcccagt gcatcaacgg caaagtgctg
1741 atcatgcacg gaggcctggt cagtgaagac ggtgtcaccg tggatgacat ccggaaaatt
1801 gagcggaatc gacaaccccc agattcaggg cccatgtgtg acctgctctg gtcagatcca
1861 cagccacaga acggggcgtc gatcagcaag cggggcgtga gctgtcagtt tgggcctgac
1921 gtcaccaagg ctttcttggg agagaacaac ctggactata tcatccgcag ccacgaagtc
1981 aaggccgagg gctacgaggt ggctcacgga ggccgctgtg tcaccgtctt ctctgcccc
2041 aactactgcg accagatggg gaacaaagcc tcctacatcc acctccaggg ctctgacct
2101 cggcctcagt tccaccagtt cacagcagtg cctcatccca acgtcaagcc catggcctat
2161 gccaacacgc tgctgcagct aggaatgatg tga
```

Reviewed and approved by site quality representative.

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.