

Certificate of Analysis

PP1 alpha

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

Item # 14-595, 14-595-K, 14-595M

Parent Lot # WAB0439

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: Untagged, recombinant, full length, human PP1 alpha expressed in *E.coli* cells. Purified using Heparin sepharose followed by Q sepharose. Purity 59% by SDS PAGE and Coomassie blue staining. MW = 37.6kDa.

Specific Activity PP1a (Parent lot# WAB0439): 12373Units/mg. A Unit releases 1nmole of phosphate min^{-1} from the phosphorylated substrate DiFMUP (6,8-difluoro-4-methylumbelliferyl phosphate Molecular Probes cat# D6567.)

Formulation: 0.63mg/ml of enzyme in 50mM Hepes/NaOH pH7.5, 300mM NaCl, 50% glycerol, 0.1mM EGTA, 1mM MnCl_2 , 0.03% Brij-35, 0.1mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Liquid at -20°C .

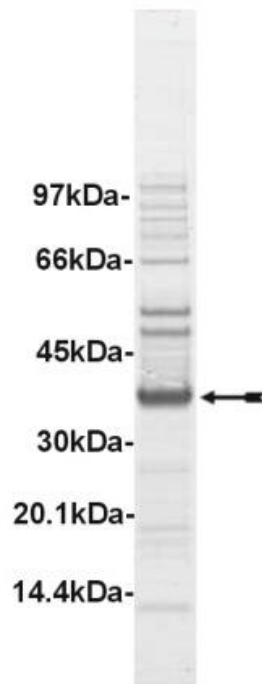
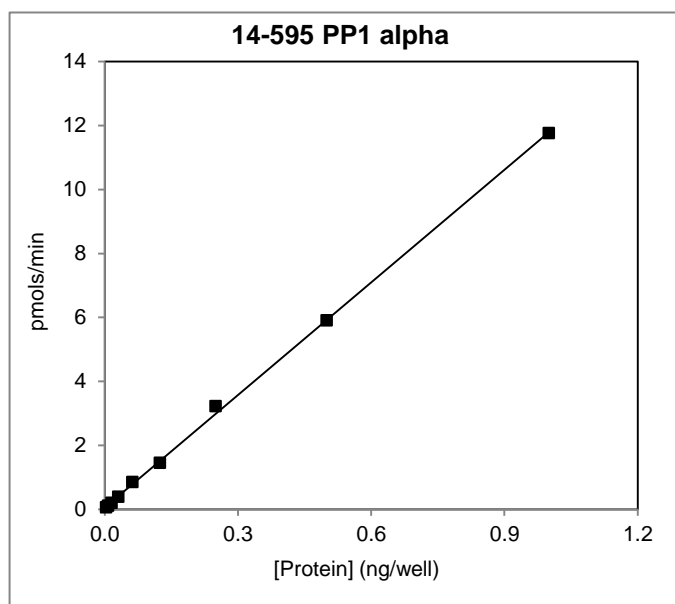
Storage and Stability: On receipt of material store at -20°C . Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

Quality Control Testing

Phosphatase Assay: Assay background was the 0 time point Fluorescence Intensity (FI) subtracted from the FI post 60 min incubation at room temperature (22°C). Quantification of FI was against a DiFMU std curve in the reaction buffer. The assay procedure followed is described overleaf.

MS Tryptic Fingerprint: Confirmed product identity as PP1 alpha with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using $3\mu\text{g}$ of active PP1 alpha.

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Phosphatase Assay Protocol

Stock Solutions:

1. 1x Reaction Buffer: 57mM Hepes pH7.2, 10mM MnCl₂, 0.167mM DTT, 0.83% (v/v) glycerol, 0.0167% (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. Add 15µl PP1a diluted in 1x reaction buffer (0.004–1.0ng well)
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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PP1 alpha Sequence Information

<u>Protein</u>	human PP1alpha
<u>Tags</u>	untagged
<u>Native sequence</u>	M1 of the recombinant protein is equivalent to M1 of PP1 alpha
<u>Accession number</u>	GenBank NM_002708. The PP1alpha protein sequence is identical to the human PP1 alpha described in GenBank NM_002708. The DNA sequence, however, is more closely related to the rabbit cDNA described in GenBank X14832.

Recombinant PP1 alpha amino acid sequence:

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1 MSDSEKLNLD SIIGRLLEVQ GSRPGKNVQL TENEIRGLCL KSREIFLSQP ILLELEAPLK
61 ICGDIHQYY DLLRLFYGG FPPESNYFLF GDYVDRGKQS LETICLLLAY KIKYPENFFL
121 LRGNHECASI NRIYGFYDEC KRRYNIKLWK TFTDCFNCLP IAAIVDEKIF CCHGGLSPDL
181 QSMEQIRIM RPTDVPDQGL LCDLLWSDPD KDVQGWGEND RGVSFTEGAE VVAKFLHKHD
241 LDLICRAHQV VEDGYEFFAK RQLVTLFSAP NYCGEFDNAG AMMSVDETLM CSFQILKPAD
301 KNKGKYGFQFS GLNPGGRPIT PPRNSAKAKK
  
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Recombinant PP1 alpha nucleotide sequence:

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1 atgtccgaca gcgagaagct caacctggac tctatcatcg ggcgctgct ggaagtgcag
61 ggctcgcggc ccggaagaa tgtgcagctg acggagaacg agatccgtgg tctgtgcctc
121 aaatcccggg agatcttct gagccagccc attctgctgg agctggaggc gcccctcaag
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481 atcgcggcca ttgtggacga gaagatattc tgctgccatg gcggcctctc ccccgacctg
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