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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 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, 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.

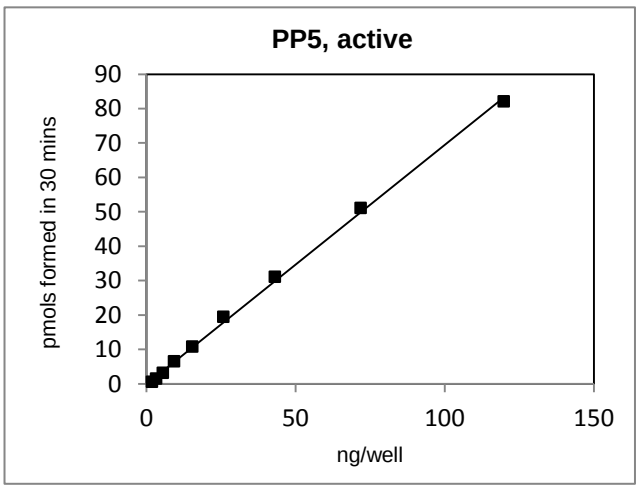
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

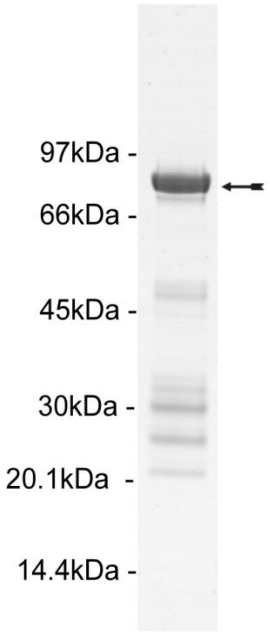
Quality Control Testing

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

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of PP5, active.



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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 **PP5** in reaction buffer and use 2.01–119.98ng 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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PP5 Sequence Information

Protein Human PP5

Tags *N*-terminal GST

Native sequence M232 of the recombinant protein is equivalent to M1 of human PP5

Accession number Genbank NM_006247

Recombinant PP5 amino acid sequence:

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  1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
 61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFQGPLG SMAMAEGERT
241 ECAEPPRDEP PADGALKRAE ELKTQANDYF KAKDYENAIK FYSQAIELNP SNAIYYGNRS
301 LAYLRTECYG YALGDATRAI ELDKKYIKGY YRRAASNMAL GKFRAALRDY ETVVKVKPHD
361 KDAKMKYQEC NKIVKQKAFE RAIAGDEHKR SVVDSLDIES MTIEDEYSGP KLEDGKVTIS
421 FMKELMQWYK DQKKLHRKCA YQILVQVKEV LSKLSTLVET TLKETEKITV CGDTHGQFYD
481 LLNIFELNGL PSETNPYIFN GDFVDRGSFS VEVILTLFGF KLLYPDHFHL LRGNHETDNM
541 NQIYGFEGEV KAKYTAQMYE LFSEVFEWLP LAQCINGKVL IMHGGLFSED GVTLDDIRKI
601 ERNRQPPDSG PMCDLLWSDP QPQNGRSISK RGVSCQFGPD VTKAFLEENN LDYIIRSHEV
661 KAEGYEVAHG GRCVTVFSAP NYCDQMGNKA SYIHLQGSDL RPQFHQFTAV PHPNVKPMAY
721 ANTLLQLGMM
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Recombinant PP5 nucleotide sequence:

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   1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaacccac tcgacttctt
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 181 ggtgatgtta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
 241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
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1501 ggtgactttg tggaccgagg ctccttctct gtagaagtga tcctcaccct tttcggcttc
1561 aagctcctgt acccagatca ctttcacctc cttcgaggca accacgagac agacaacatg
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1621 aaccagatct acggtttcga gggtgaggtg aaggccaagt acacagccca gatgtacgag
1681 ctctttagcg aggtgttcga gtggctcccg ttggcccagt gcatcaacgg caaagtgctg
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2161 gccaacacgc tgctgcagct aggaatgatg tga
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Reviewed and approved by site quality representative.