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Certificate of Analysis

PTP MEG1, active

(Recombinant enzyme expressed in *E. coli* cells)
Item # 14-642
Lot # 1987060

Product Description: *N*-terminal GST-tagged, recombinant, human PTP MEG1, amino acids 423–end, expressed in *E. coli* cells. Purified using glutathione agarose. Purity 56% by SDS-PAGE and Coomassie blue staining. MW = 83.1kDa.

Specific Activity (lot# 1987060): 239U/mg, where one unit of PTP MEG1 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: 1.09mg/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.

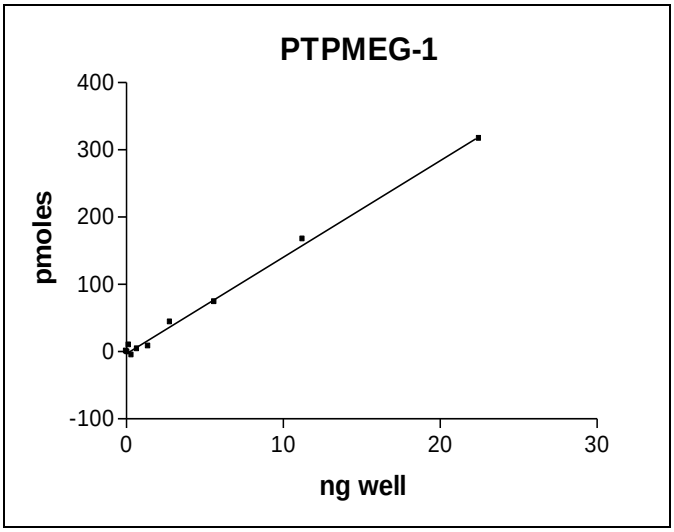
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

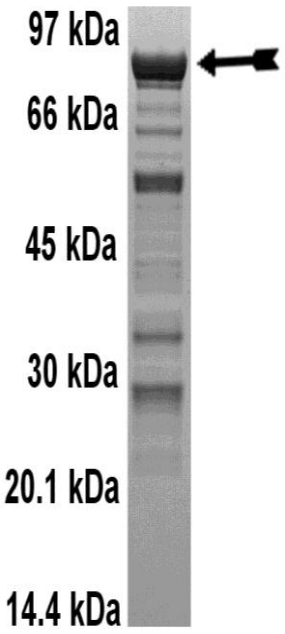
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Phosphatase Assay: 0–11ng 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 PTP MEG1 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 active PTP MEG1.

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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 **PTP-MEG1** in reaction buffer and use 0–11ng 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.

Preparation of DiFMU Standard Calibration Curve

1. Prepare a series of standards according to the table below:

Volume of 100µM DiFMU (product)	Volume of 100µM DiFMUP (substrate)	pmoles DiFMU per well	Represents Substrate Conversion of:
0µl	100µl	0	0%
1µl	99µl	25	1%
2µl	98µl	50	2%
3µl	97µl	75	3%
4µl	96µl	100	4%
5µl	95µl	125	5%
8µl	92µl	200	8%
12µl	88µl	300	12%

2. Add 25µl of each prepared standard to triplicate wells and read fluorescence intensity using an appropriate reader (Excitation 358nm, Emission 455nm).

Note: Substrate conversion above 15% introduces the possibility of skewing the curve so that calculated specific activity will be less than actual specific activity. This substrate limitation kinetic varies with each enzyme and may be determined by plotting product formed per unit time versus enzyme concentration. At low enzyme concentrations, the rate of product formation will be proportional to the enzyme concentration used. This relationship plateaus at higher enzyme concentrations.

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PTP MEG1 Sequence Information

Protein	Human PTPMEG-1
Tags	N-Terminal GST
Native sequence	M227 of the recombinant protein is equivalent to M423 of human PTPMEG-1
Accession number	GenBank NM_002830

Recombinant PTPMEG-1 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 LVPRGSMVHT SPSEVFVNQR
241 SPSSTQANSI VLESSPSQET PGDGKPPALP PKQSKKNSWN QIHYSHSQQD LESHINETFD
301 IPSSPEKPTP NGGIPHDNLV LIRMKPDENG RFGFNVKGGY DQKMPVIVSR VAPGTPADLC
361 VPRLNEGDQV VLINGRDIAE HTHDQVVLFI KASCERHSGE LMLLVRPNAV YDVVEEKLEN
421 EPDFQYIPEK APLDSVHQDD HSLRESMIQL AEGLITGTVL TQFDQLYRKK PGMTMSCAKL
481 PQNISKNRYR DISPYDATRV ILKGNEDYIN ANYINMEIPS SSIINQYIAC QGPLPHTCTD
541 FWQMTWEQGS SMVVMLTTQV ERGRVKCHQY WPEPTGSSSY GCYQVTCHSE EGNTAYIFRK
601 MTLFNQEKNE SRPLTQIQYI AWPDHGVPDD SSDFLDFVCH VRNKRAGKEE PVVVHCSAGI
661 GRTGVLITME TAMCLIECNQ PVYPLDIVRT MRDQRAMMIQ TPSQYRFVCE AILKVYEEGF
721 VKPLTTSTNK
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Recombinant PTPMEG-1 nucleotide sequence:

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   1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaacccac tcgacttctt
  61 ttggaatatc ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
 121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
 181 ggtgatgtta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
 241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
 301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
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 421 acatatttaa atggtgatca tgtaacccat cctgacttca tgttgtatga cgctcttgat
 481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
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 661 ctggttccgc gtggatccat ggttcatact tccccaagcg aagtgtttgt aaatcagaga
 721 tctccgtcat caacacaagc taatagcatt gttctggaat catcaccatc acaagagacc
 781 cctggagatg ggaagcctcc agctttacca cccaaacagt caaagaaaaa cagttggaac
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 961 ctaatcagaa tgaaacctga tgaaaatggg aggtttggat tcaatgtaaa gggaggatat
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1621 ttttggcaga tgacttggga acaaggctcc tctatggttg taatgttgac cacacaagtt
1681 gaacgtggca gagttaaatg tcaccaatat tggccagaac ccacaggcag ttcatcttat
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1741 ggatgctacc aagttacctg ccactctgaa gaaggaaaca ctgcctatat cttcaggaag
1801 atgaccctat ttaaccaaga gaaaaatgaa agtcgtccac tcactcagat ccagtacata
1861 gcctggcctg accatggagt ccctgatgat tcgagtgact ttctagattt tgtttgtcat
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2101 acacctagtc aatacagatt tgtatgtgaa gctattttga aagtttatga agaaggcttt
2161 gttaaaccct taacaacatc aacaaataaa taa
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Reviewed and approved by site quality representative.