

Certificate of Analysis

PTP MEG2

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

Item # 14-592, 14-592-K, 14-592M

Parent Lot # 2327880

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 human PTP MEG2, amino acid residues 283–end, expressed in *E.coli* cells. Purified using glutathione-agarose. Purity 81.2% by SDS-PAGE and Coomassie blue staining. MW: 62.5kDa

Specific Activity (Parent lot# 2327880): 50923U/mg where one unit of PTP MEG2 activity is defined as the release of 1nmole of phosphate/min from the phosphorylated substrate DiFMUP (6,8-difluoro-4-methylumbelliferyl phosphate; Molecular Probes cat# D6567.)

Formulation: 0.466mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 10% glycerol, 0.1mM EGTA, 0.03% Brij-35, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

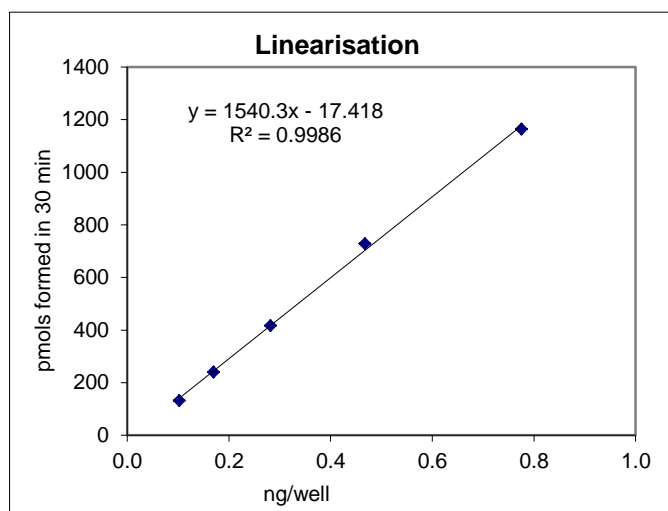
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

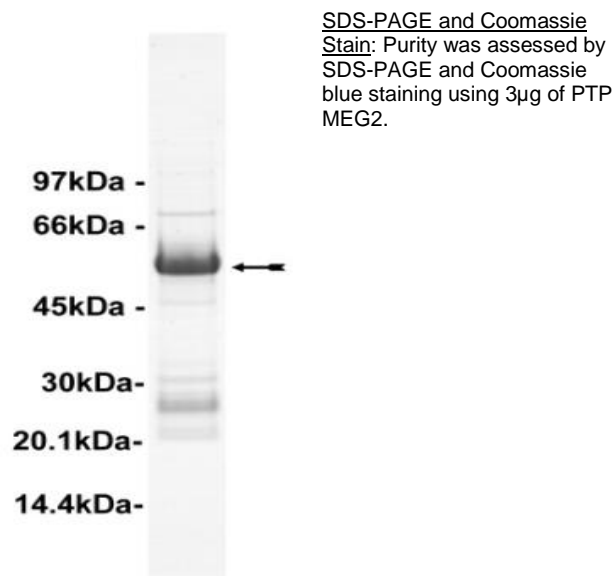
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Phosphatase Assay: 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 on page two.



MS Tryptic Fingerprint: Confirmed identity as PTP MEG2 with the translated sequence listed on page three.



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Phosphatase Assay Protocol

Stock Solutions:

1. 1x Reaction Buffer: 50mM Hepes pH7.2, 10mM MgCl₂, 1mM DTT, 0.1% BSA.
2. 250µM DiFMUP in 1x reaction buffer.
3. 100µM DiFMUP in 1x reaction buffer.
4. 100µM DiFMU in 1x reaction buffer.

Assay Procedure:

1. Add 15µl **PTP MEG2** diluted in 1x reaction buffer (0.04–1.4ng well⁻¹)
2. Add 10µl 250uM DIFMUP prepared in 1x Reaction Buffer.
3. Incubate for 60mins at 22°C
4. Read fluorescence intensity using an appropriate reader (Excitation 358nm, Emission 455nm).
5. Subtract the 0 time and then the 0 enzyme value from each Fluorescence Intensity reading and calculate the enzyme activity by conversion to pmoles product formed using a 6,8-difluor-7-hydroxy-4-methylcoumarin (DIFMU) standard calibration curve.

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PTP MEG2 Sequence Information

Protein	PTP MEG2
Tags	N-terminal GST
Native sequence	P230 of the recombinant protein is equivalent to P283 of human PTP MEG2
Accession number	GenBank NM_002833

Recombinant PTP MEG2 amino acid sequence:

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1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQ SMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAI PQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSPEFP HAMTIQELVD
241 YVNARQKQGI YEEYEDIRRE NPVGT FHC SM SPGNLEKNRY GDVPCLDQTR VKLTKRS GHT
301 QTDYINASF M DGYKQKNAYI GTQGPLENTY RDFWLMVWEQ KVLVIVMTTR FEEGRRKCG
361 QYWPLEKDSR IRFGFLT VTN LGVENMNH YK KTTLEIH NTE ERQKRQVTHF QFLSWPDYGV
421 PSSAASLIDF LRVVRNQQSL AVSNMGARSK GQCPEPPIV HCSAGIGRTG TFCSLDICLA
481 QLEELGTLNV FQTVSRMRTQ RAFSIQTPEQ YYFCYKAILE FAEKEGMVSS GQNLLAVESQ
  
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Recombinant PTP MEG2 nucleotide sequence:

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1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccac tcgacttctt
61 ttggaatatc ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatatgat
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1621 taa
  
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