

Certificate of Analysis

RPTPmu, active

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

Item # 14-780, 14-780-K, 14-780M

Parent Lot # D7HN087N

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 RPTPmu, amino acids 879–1184, expressed in *E. coli* cells. Purified using glutathione agarose. Purity 95.5% by SDS-PAGE and Coomassie blue staining. MW = 61.9kDa.

Specific Activity (Parent lot# D7HN087N): 32412 U/mg, where one unit of RPTPmu, 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: 0.93mg/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.

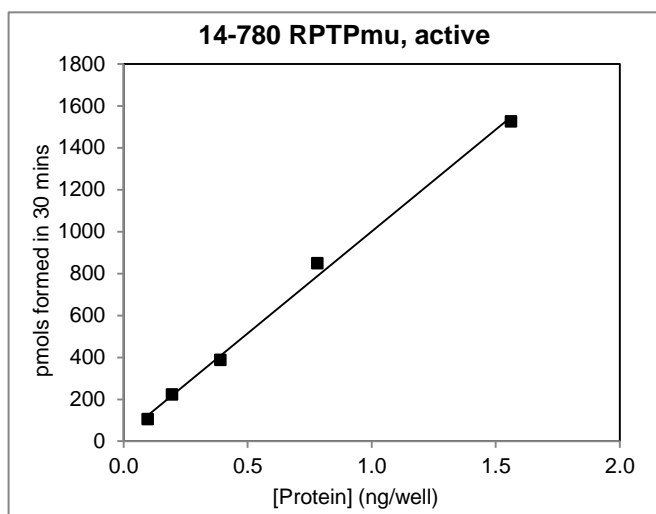
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

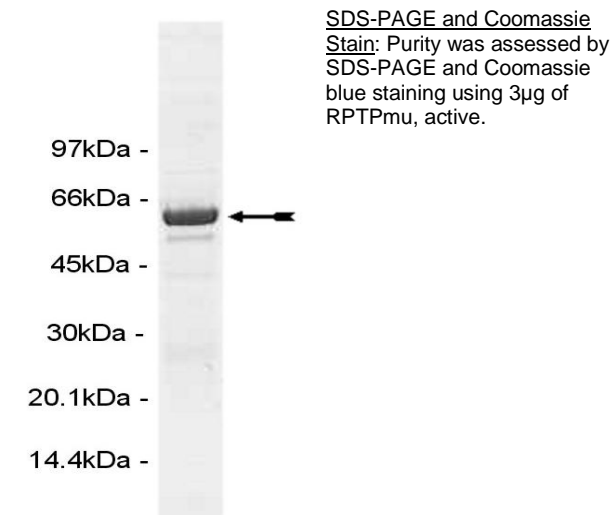
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Phosphatase Assay: 0.10–0.78ng 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 RPTPmu with the translated sequence listed on page three.



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Phosphatase Assay Protocol

Stock Solutions:

1. **Reaction Buffer:** 32mM Hepes pH7.2, 50mM NaCl, 2.5mM EDTA, 0.17mM DTT, 0.83 (v/v)% glycerol, 0.017 (w/v)% BSA, 0.002% Brij-35.
2. 500 μ M DiFMUP (Molecular Probes Catalogue# D6567) in water.
3. 100mM sodium orthovanadate.
4. 500 μ M DiFMU (Molecular Probes Catalogue# D6566) in water for the calibration curve.

Assay Procedure:

1. Dilute **RPTPmu** in reaction buffer and use 0.10–1.56ng in 15 μ l per assay point.
2. Add 10 μ l DiFMUP 500 μ M stock solution (200 μ M final assay concentration).
3. Incubate for 30 minutes at room temperature.
4. Stop the reaction by adding 5 μ 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.

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RPTPmu Sequence Information

<u>Protein</u>	Human RPTPmu
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	A230 of the recombinant protein is equivalent to A879 of human RPTPmu
<u>Accession number</u>	Genbank NM_002845

Recombinant RPTPmu amino acid sequence:

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1 MSPILGYWKI KGLVQPTRL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQ SMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSR IA YSKDFETLKV
121 DFLSKLP EML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAI PQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSPEFA IRVADLLQHI
241 TQMKCAEG YG FKEEYESFFE QQSAPWDSAK K DENRMKNRY GNIIAYDHSR VRLQTIEGDT
301 NSDYING NYI DGYHRPNHYI ATQGP MQETI YDFWRMVWHE NTASIIMVTN LVEVGRVKCC
361 KYWPDDEI Y KDIKVTLIET ELLAEYVIRT FAVEKRGVHE IREIRQFHFT GWPDHGV P YH
421 ATGLLG FVRQ VKSKSPPSAG PLVHCSAGA GRTGCFIVID IMLDMAEREG VVDIYN CVRE
481 LRSRRV NMVQ TEEQVVF IHD AILEACLGD TSVPASQVRS LYYDMNKLD P QTNSS
  
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Recombinant RPTPmu nucleotide sequence:

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1 atgtccccta tactaggtta ttgga a aatt aagggccttg tgcaaccac tcgacttctt
61 ttggaat atc ttgaagaaa atatgaagag catttgatg agcgcgatga aggtgataaa
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181 ggtgatg tta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
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1561 ctgtattatg acatgaacaa actggatcca cagacaaact caagctaa
  
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