

Certificate of Analysis

TMDP, active

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

Item # 14-767, 14-767-K, 14-767M

Parent Lot # D7AN015N

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 TMDP, full length, expressed in *E. coli* cells. Purified using glutathione agarose.

Purity 99% by SDS-PAGE and Coomassie blue staining. MW = 49kDa.

Specific Activity (Parent lot# D7AN015N): 2115U/mg, where one unit of TMDP 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: 4.35mg/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.

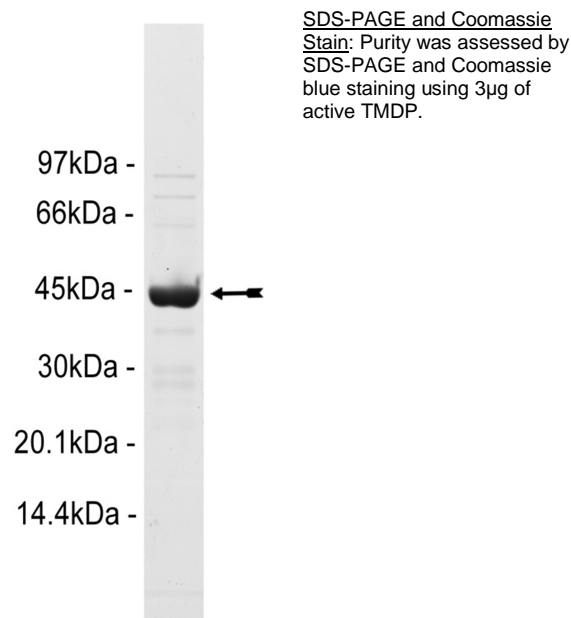
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

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

Quality Control Testing

Phosphatase Assay: 0.1–1.6ng 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 TMDP with the translated sequence listed on page three.



Certificate of Analysis

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. 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 **TMDP** in reaction buffer and use 0.1–1.6ng 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.

Certificate of Analysis

TMDP Sequence Information

<u>Protein</u>	Human TMDP
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	M230 of the recombinant protein is equivalent to M1 of human TMDP
<u>Accession number</u>	GenBank NM_016364

Recombinant TMDP amino acid sequence:

```

1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQSMa IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIPOID KYLKSSKYIA WPLQGWQATF GGDHPPKSD LVPRGSPEFM DSLQKQDLRR
241 PKIHGAVQAS PYQPPTLASL QRLLWVRQAA TLNHIDEVWP SLFLGDAYAA RDKSKLIQLG
301 ITHVVAAG KFQVDTGAKF YRGMSEYYG IEADDNPFFD LSVYFLPVAR YIRAALSVPQ
361 GRVLVHCAMG VRSRATLVLA FLMIYENMTL VEAIQTVQAH RNICPNSGFL RQLQVLDNRL
421 GRETGRF
  
```

Recombinant TMDP nucleotide sequence:

```

1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccac tcgacttctt
61 ttggaatata ttgaagaaaa atatgaagag catttgatg agcgcgatga aggtgataaa
121 tggcgaaaaca aaaagttaga attgggtttg gaggttccca atcttcctta ttatattgat
181 ggtgatgtta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcgggtttg
301 gatattagat acgggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
361 gattttctta gcaagctacc tgaatgctg aaaatgttcg aagatcgttt atgtcataaa
421 acatatttaa atggtgatca tgtaaccat cctgacttca tgttgtatga cgctcttgat
481 gttgttttat acatggacc ccaatgtgct gatgcttcc caaattagt ttgttttaaa
541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
661 ctggattccgc gtggatcccc ggaattcatg gactcactgc agaagcagg cctccggagg
721 cccaagatcc atggggcagt ccaggcatct ccctaccagc cgccacatt ggcttcgctg
781 cagcgtttgc tgtgggtccg tcaggctgcc aactgaacc atatcgatga ggtctggccc
841 agcctcttcc tgggagatgc gtacgcagcc cgggacaaga gcaagctgat ccagctggga
901 atcaccacag ttgtgaatgc cgctgcaggc aagtccagg tggacacagg tgccaaattc
961 taccgtggaa tgtccctgga gtactatggc atcgaggcgg atgacaacc cttcttcgac
1021 ctcagtgtct actttctgcc tgttgcctga tacatccgag ctgccctcag tgttcccaa
1081 ggccgcgtgc tggtagactg tgccatgggg gtaagccgct ctgccacact tgcctcggcc
1141 ttctctcatga tctatgagaa catgacgctg gtagaggcca tccagacggt gcaggcccac
1201 cgcaatatct gccctaactc aggtctcttc cggcagctcc aggttctgga caaccgactg
1261 gggcgggaga cggggcggtt ctga
  
```

Reviewed and approved by site quality representative.

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

© 2014 Eurofins Pharma Discovery Services UK Limited is an independent member of Eurofins Discovery Services.