

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, 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: 4.35mg/ml of enzyme in 50mM Tris/HCl pH8.5, 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.

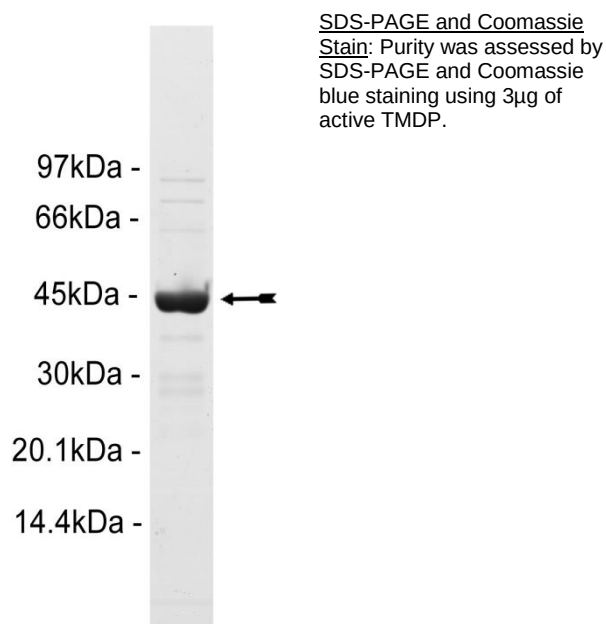
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

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

Quality Control Testing

Phosphatase Assay: 0.1–1.6ng 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.



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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. 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.

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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:

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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 KRIEAIQID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LVPRGSPEFM  DSLQKQDLRR
241 PKIHGAVQAS  PYQPPTLASL  QRLLWVRQAA  TLNHIDEVWP  SLFLGDAYAA  RDKSKLIQLG
301 ITHVNAAG  KFQVDTGAKF  YRGMSLEYYG  IEADDNPFFD  LSVYFLPVAR  YIRAALSPVQ
361 GRVLVHCAMG  VRSATLVLA  FLMIYENMTL  VEAIQTVQAH  RNICPNSGFL  RQLQVLDNRL
421 GRETGRF

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Recombinant TMDP nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tgcacttctt
61  ttggaatatc  ttgaagaaaa  atatgaagag  catttgatat  agcgcgatga  aggtgataaa
121  tggcgaaaca  aaaagtgtga  attgggtttg  gagtttccca  atcttcctta  ttatatgat
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421  acatatttta  atggtgatca  tgtaacccat  cctgacttca  tgttgatatg  cgctcttgat
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1261  gggcgggaga  cggggcggtt  ctga

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