

Certificate of Analysis

TCPTP, active

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

Item # 14-646, 14-646-K, 14-646M

Parent Lot # 2067980

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: Untagged, recombinant, human TCPTP, amino acids 1–341, expressed in *E. coli* cells. Purified using ion exchange chromatography. Purity 96.8% by SDS-PAGE and Coomassie blue staining. MW = 39.7kDa.

Specific Activity (Parent lot# 2067980): 23826U/mg, where one unit of TCPTP, 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: 0.187mg/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.

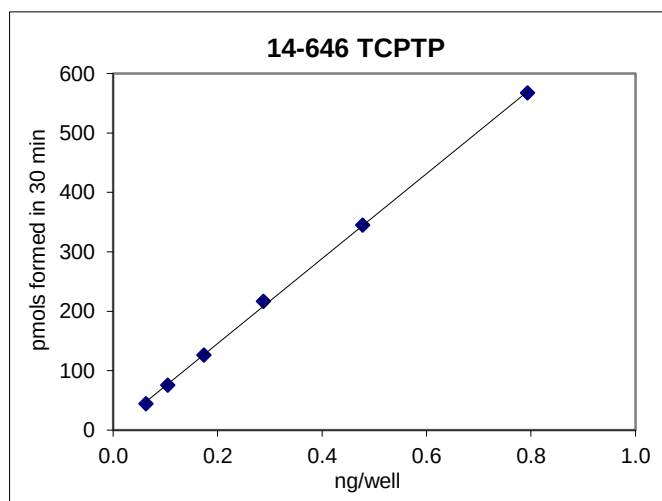
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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**

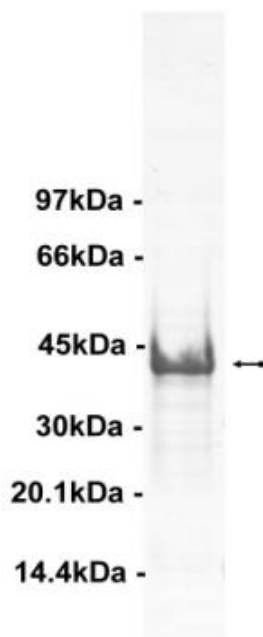
Quality Control Testing

Phosphatase Assay: 0.06–0.8ng 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 TCPTP 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 TCPTP, active.



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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. 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 TCPTP in reaction buffer and use **0.06–0.8ng** 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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TCPTP Sequence Information

<u>Protein</u>	Human TCPTP
<u>Tags</u>	Untagged
<u>Native sequence</u>	M1 of the recombinant protein is equivalent to M1 of human TCPTP
<u>Accession number</u>	GenBank NM_080422

Recombinant TCPTP amino acid sequence:

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1  MPTTIEREFE ELDTQRRWQP LYLEIRNESH DYPHRVAKFP ENRNRNRYRD VSPYDHSRVK
61  LQNAENDYIN ASLVDIEEAQ RSYILTQGPL PNTCCHFWMV VWQQKTKAVV MLNRIVEKES
121 VKCAQYWPTD DQEMLFKETG FSVKLLSESV KSYITVHLLQ LENINSGETR TISHFHYTTW
181 PDFGVPESPA SFLNLFKVR ESGLNPDHG PAVIHCSAGI GRSGETFSLVD TCLVLMEKGD
241 DINIKQVLLN MRKYRMGLIQ TPDQLRFSYM AIIEGAKCIK GDSSIQRWK ELSKEDLSPA
301 FDHSPNKIMT EKYNNGNRIGL EEEKLTGDR TGLSSKMQDT M

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Recombinant TCPTP nucleotide sequence:

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1  atgcccacca ccatcgagcg ggagttcgaa gagttggata ctcagcgctcg ctggcagccg
61  ctgtacttgg aaattcgaaa tgagtcccat gactatcctc atagagtggc caagtttcca
121 gaaaacagaa atcgaaacag atacagagat gtaagcccat atgacacag tcgtgtttaa
181 ctgcaaaatg ctgagaatga ttatattaat gccagtttag ttgacataga agaggcacia
241 aggagttaca tcttaacaca ggtgccactt cctaacacat gctgccattt ctggcttatg
301 gtttggcagc agaagaccaa agcagttgtc atgctgaacc gcattgtgga gaaagaatcg
361 gttaaatgtg cacagtactg gccaacagat gaccaagaga tgctgtttaa agaaacagga
421 ttcagtgtga agctcttgtc agaagatgtg aagtcgtatt atacagtaca tctactacaa
481 ttagaaaata tcaatagtgg tgaaaccaga acaatatctc actttcatta tactacctgg
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601 gaatctggct ccttgaaccc tgaccatggg cctgcggtga tccactgtag tgcaggcatt
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721 gatattaaca taaaacaagt gttactgaac atgagaaaat accgaatggg tcttattcag
781 accccagatc aactgagatt ctcatatcat gctataatag aaggagcaaa atgtataaag
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901 tttgatcatt caccaaaca aataatgact gaaaaataca atgggaacag aataggtcta
961 gaagaagaaa aactgacagg tgaccgatgt acaggacttt cctctaaaat gcaagataca
1021 atgtaa

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