



Product Information

T-Cell Protein Tyrosine Phosphatase Human, recombinant

Product Number **T 1196**
Storage Temperature $-70\text{ }^{\circ}\text{C}$

EC# 3.1.3.48
Synonym: TC-PTP

Product Description

T-Cell Protein Tyrosine Phosphatase is a human recombinant protein, which contains a N-terminal histidine tag and is expressed in *E. coli*. This product is a C-terminal truncated form of the 45 kDa variant (TC45) containing amino acids 1-352.¹

T-Cell Protein Tyrosine Phosphatase (TC-PTP) belongs to the non-receptor type phosphatases. It acts with the protein tyrosine kinases to govern the level of tyrosine phosphorylation, thus regulating diverse physiological processes. TC-PTP was originally cloned from T-cells, but it is now known to be expressed in many human tissues. It contains a conserved catalytic domain of 240 amino acids located at its N-terminus and a non-catalytic C-terminal domain that varies in size, hydrophobicity, and function due to alternative splicing.

TC-PTP acts on the signaling pathway initiated by epidermal growth factor receptor and is important in the hematopoietic system. It is expressed in higher amounts in lymphoid lineage cells.

The product is supplied as a solution in 50 mM Tris, pH 7.5, with 2 mM EDTA, 5 mM DTT, 100 mM NaCl, 0.01% BRIJ 35, and 50% Glycerol

Purity: Minimum 90% (SDS-PAGE)

Specific activity: minimum 14,000 units/mg protein (Bradford)

Unit definition: One unit will hydrolyze 1.0 nmole of p-nitrophenyl phosphate per minute at pH 7.0 at $30\text{ }^{\circ}\text{C}$.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

This product ships on dry ice and long term storage at $-70\text{ }^{\circ}\text{C}$ is recommended. Avoid freeze and thaw cycles.

Preparation Instructions

1. 2x Reaction Buffer: 50 mM imidazole, pH 7.0, with 0.2 mg/ml BSA, 10 mM DTT, 100 mM NaCl, 5 mM EDTA, and 100 mM pNPP (p-nitrophenyl phosphate, Product No. N 3254)
2. Enzyme Dilution Buffer: 10 mM imidazole, pH 7.0
3. Stop solution: 0.2 M NaOH solution
4. Water bath set to $30\text{ }^{\circ}\text{C}$.
5. Spectrophotometer capable of recording the absorbance at 405 nm and appropriate cuvettes.

Procedure

The activity of TC-PTP is determined by measuring the dephosphorylation of a synthetic substrate, pNPP.

1. Dilute the sample 100-fold with the Enzyme Dilution Buffer.
2. Pipette 100 μl of the diluted sample into an Eppendorf[®] tube. Each sample should be run in duplicate and a blank with the Enzyme Dilution Buffer alone should also be run.
3. Start the reaction with the addition of 100 μl of the 2x Reaction Buffer.
4. Mix gently and incubate for 10 minutes at $30\text{ }^{\circ}\text{C}$.
5. Stop the reaction with the addition of 800 μl of 0.2 M NaOH.
6. Measure the absorbance difference at 405 nm (ΔA_{405}) between the samples and the blank.

Results

Unit definition: One unit is the amount of TC-PTP that will hydrolyze 1.0 nmole of p-nitrophenyl phosphate per minute at pH 7.0 at 30 °C.

$$\begin{array}{l} \text{Activity of} \\ \text{original sample} \\ \text{(nmole/min/ml)} \end{array} = \frac{\Delta A_{405} \times d \times v \text{ (assay)}}{0.018 \times t \times v \text{ (sample)}}$$

0.018 = $\epsilon_{\mu\text{M}}$ (micromolar extinction coefficient of pNPP under alkaline conditions)

t – reaction time (minutes)

d – dilution factor of original sample

v (assay) – volume of assay = 1 ml

v (sample) – volume (ml) of diluted sample used in assay

References

1. Hao, L., et al., J. Biol. Chem., **272**, 29322 (1997).
2. Ibarra-Sanchez, M de J, et al., Semin. Immunol., **12**, 379 (2000).
3. Mustelin, T., et al., Cell Signal., **11**, 637 (1999).

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