T-Cell Protein Tyrosine Phosphatase (TC PTP)

1-800-632-7799
info@neb.com
www.neb.com

P0752S 00412081408 1

P0752S

200 units **10,000 U/ml** **Lot: 0041208**

RECOMBINANT **Store at –70°C Exp: 8/14**

Description: T-Cell Protein Tyrosine Phosphatase (TC PTP) is a phospho tyrosine-specific protein phosphatase. It is a truncated form of the human T-Cell protein tyrosine phosphatase (residues 1-317) which lacks a C-terminal regulatory domain (1,2).

Recognition Determinants: The truncated form of TC PTP preferentially dephosphorylates substrates with acidic residues located on the N-terminal side of phosphotyrosine (positions -3 and/or -4) with special preference to position -3, while basic residues in the same positions act as negative determinants.

New Reaction Buffer

The full length TC PTP shows a similar substrate specificity but not identical to that of the truncated enzyme. The dephosphorylation efficiency of the full length enzyme is dramatically impaired with peptide substrates by a substantial increase in K_m values. It was suggested that the C-terminal regulatory domain of TC PTP plays a role in altering the affinity of the enzyme towards its phosphotyrosyl targets (3).

Source: Isolated from a strain of *E. coli* that carries a clone expressing the T-Cell protein tyrosine phosphatase under the control of the T7 expression system (kindly provided by Dr. D. Bardford).

Applications: TC PTP can be used to release phosphate groups specifically from phospho-tyrosine residues in proteins. Note that different proteins are dephosphorylated at different rates.

Supplied in: 100 mM NaCl, 50 mM HEPES (pH 7.0 @ 25°C), 2 mM Na_2EDTA, 5 mM DTT, 0.01% Brij 35 and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer for Protein Tyrosine Phosphatase (PTP)
Reaction Conditions: 1X NEBuffer for PTP
Incubate at 30°C.

1X NEBuffer for PTP:
50 mM Tris-HCl
100 mM NaCl
2 mM Na_2EDTA
5 mM DTT
0.01% Brij 35
pH 7.5 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme that hydrolyzes 1nmol of *p*-Nitrophenyl Phosphate (50 mM) (NEB #P0757) in 1 minute at 30°C in a total reaction volume of 50 µl.

Specific Activity: ~40,000 units/mg

Molecular Weight: 37 kDa

Purity: TC PTP has been purified to > 95% homogeneity as determined by SDS-PAGE and Coomassie Blue staining.

Quality Control Assays

Protease Activity: After incubation of 100 units of TC PTP with a standardized mixture of proteins for 2 hours at 30°C, no proteolytic activity could be detected by SDS-PAGE.

Serine-Threonine Phosphatase Activity: After incubation of 100 units of TC PTP with phosphorylated myelin basic protein (10 µM with respect to phosphate) for 2 hours, no serine/threonine phosphatase activity could be detected by release of radioactivity.

Myelin basic protein was phosphorylated exclusively on serine/threonine residues with cAMP-dependent Protein Kinase.

Heat Inactivation: 65°C for one hour

Notes On Use: Avoid freeze/thaw cycles. Can be stored for 2 weeks or less at –20°C.

(See other side)

CERTIFICATE OF ANALYSIS

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CERTIFICATE OF ANALYSIS

The following information can be used as suggested initial conditions for dephosphorylation of proteins with TC PTP.

1 unit of TC PTP removes ~100% of phosphates (0.5 nmol) in phosphorylated myelin basic protein (phospho-MyBP, 18.5 kDa) in 30 minutes in a 50 µl reaction. The concentration of phospho-MyBP is 10 µM with respect to phosphate.

The Protein Tyrosine Phosphatase (PTP) activity of TC PTP is assessed on MyBP phosphorylated exclusively on tyrosine residues with Abl Protein Tyrosine Kinase.

Optimal incubation times and enzyme concentrations must be determined empirically for each particular substrate.

TC PTP is inhibited by vanadate (2).

If the source of phosphorylated protein is a crude extract of cells or tissue, it is very important to include the appropriate protease inhibitors in the lysis buffer and to use shorter incubation time for dephosphorylation.

The following levels of inhibition of TC PTP (1 unit) are found when the reaction buffer is supplemented with:

1 mM Sodium Orthovanadate (NEB #P0758) (3)	100%
50 mM Sodium Fluoride (NEB #P0759)	no
1% Triton X-100	no
0.4% Nonidet P-40	no
0.025% Tween 20	no
0.5 M NaCl	10%
ATP Mix (10 mM $MgCl_2$, 0.1 mM ATP)	no
Protease inhibitor cocktail*	10%

*Pepstatin A, leupeptin and aprotinin, 10 µg/ml each, 0.5 mM PMSF and 1 mM benzamidine.

References:

1. Cool, D.E. et al. (1989) *Proc. Natl. Acad. Sci. USA* 86, 5257–5261.
2. Zander, N.F. et al. (1991) *Biochemistry* 30, 6964–6970.
3. Ruzzene, M. et al. (1993) *Eur. J. Biochem.* 211, 289-295.
4. Gordon, J.A. (1991) *Methods in Enzymology* 201, 477–482.

Companion Products:

NEBuffer for Protein Tyrosine Phosphatases (PTP)
#B0760S

Sodium Orthovanadate
#P0758S

p-Nitrophenyl Phosphate (PNPP)
#P0757S

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