Protein Phosphatase 1 (PP1)

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

P0754S 013131215121

P0754S

100 units **2,500 U/ml** **Lot: 0131312**

RECOMBINANT **Store at –70°C** **Exp: 12/15**

Description: Protein Phosphatase 1 (PP1) is a Mn^{2+}-dependent protein phosphatase with activity towards phosphoserine/threonine residues. It consists of the 330 amino-acid catalytic subunit of the α-isoform of type 1 protein phosphatase from rabbit skeletal muscle (1,2). Recombinant PP1 shows some activity towards phosphotyrosine residues (3,4).

Source: Isolated from a strain of *E. coli* that carries the coding sequence for rabbit skeletal muscle PP1 under the control of the *trp-lac* hybrid promoter (kindly by Dr. E.Y.C. Lee) (1,2).

New Reaction Buffer

Supplied in: 200 mM NaCl, 50 mM HEPES (pH 7.0 @ 25°C), 1 mM $MnCl_2$, 0.1 mM EGTA, 2.5 mM dithiothreitol, 0.025% Tween-20 and 50% glycerol. **Store at –70°C**

Applications: PP1 can be used to release phosphate groups from phosphorylated serine, threonine and tyrosine residues in proteins. Note that different proteins are dephosphorylated at different rates.

Reagents Supplied with Enzyme:
10X NEBuffer for Protein MetalloPhosphatases (PMP)
10X $MnCl_2$ (10 mM)

Reaction Conditions: 1X NEBuffer for PMP, supplemented with 1 mM $MnCl_2$.
Incubate at 30°C.

1X NEBuffer for PMP:
50 mM HEPES
100 mM NaCl
2 mM DTT
0.01% Brij 35
pH 7.5 @ 25°C

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

Specific Activity: ~ 31,250 units/mg.

Molecular Weight: 37.5 kDa.

Purity: PP1 has been purified to > 90% homogeneity as determined by SDS-PAGE and Coomassie Blue staining.

Quality Assurance: PP1 contains no detectable protease activity.

Quality Control Assays

Protease Activity: After incubation of 50 units of PP1 with a standardized mixture of proteins for 2 hours at 30°C, no proteolytic activity could be detected by SDS-PAGE.

Heat Inactivation: 65°C for one hour.

Notes On Use: Avoid freeze/thaw cycles. Can be stored for 1 week or less at –20°C.

The following information can be used as suggested initial conditions for dephosphorylation of proteins with PP1.

0.1 unit of PP1 removes ~100% of phosphates (0.5 nmol) from phosphoserine/threonine residues in phosphorylase *a* as well as 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 Serine/threonine Phosphatase (PSP) activity of PP1 is assessed on phosphorylase *a* phosphorylated on a serine residue with phosphorylase kinase, and also on MyBP phosphorylated on serine/threonine residues with cAMP-dependent Protein Kinase.

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

(See other side)

CERTIFICATE OF ANALYSIS

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(See other side)

CERTIFICATE OF ANALYSIS

It has been found that bacterially expressed catalytic subunits of the α- and γ-isoforms of type 1 protein phosphatase can dephosphorylate phosphotyrosine residues in proteins (3,4). We assessed the Protein Tyrosine Phosphatase (PTP) activity of PP1 on MyBP phosphorylated on tyrosine residues with Abl Protein Tyrosine Kinase. We have measured the PTP activity as high as 10–30% of the PSP activity measured on MyBP phosphorylated on serine/threonine residues.

PP1 has been shown to be active on phosphorylated histidine residues (5).

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 PP1 (0.1 unit) are found when the reaction buffer is supplemented with:

1 µM Protein Phosphatase Inhibitor 2 (NEB #P0755)	100%
10 µM okadaic acid	85%
0.1 µM microcystin-LR	100%
10 mM Sodium Orthovanadate (6) (NEB #P0758)	95%
50 mM Sodium Fluoride (NEB #P0759)	40%
50 mM Na_2 EDTA	95%
1% Triton X-10	5%
0.4% Nonidet P-40	no
0.5 M NaCl	no
Protease Inhibitor Cocktail*	no

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

References:

1. Bai, G. et al. (1988) *FASEB Journal* 2, 3010–3016.
2. Zhang, Z. et al. (1992) *J. Biol. Chem.* 267, 1484–1490.
3. Barshevsky, T. and Roberts, R.J. (1997) *The NEB Transcript* 8, No. 2, 14.
4. MacKintosh, C. et al. (1996) *FEBS Letters* 397, 225–238.
5. Kim, Y. et al. (1993) *J. Biol. Chem.* 268, 18513–18518.
6. Gordon, J.A. (1991) *Methods in Enzymology* 201, 477–482.

Companion Products:

NEBuffer Pack for Protein MetalloPhosphatases
#B0760S

Sodium Orthovanadate
#P0758S

Sodium Fluoride
#P0759S

p-Nitrophenyl Phosphate (PNPP)
#P0757S

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