Lambda Protein Phosphatase (Lambda PP)

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

P0753S 013141116111

P0753S

20,000 units **400,000 U/ml** **Lot: 0131411**
RECOMBINANT **Store at –80°C** **Exp: 11/16**

Description: Lambda Protein Phosphatase (Lambda PP) is a Mn^{2+}-dependent protein phosphatase with activity towards phosphorylated serine, threonine and tyrosine residues. It is the 221 amino-acid product of the ORF221 open reading frame on bacteriophage lambda (1,2).

Source: Isolated from a strain of *E. coli* that carries the bacteriophage lambda ORF221 open reading frame under the control of a T7 expression system (kindly provided by Dr. D. Barford) (2).

Note Storage Temperature Change

@ 25°C), 0.1 mM $MnCl_2$, 0.1 mM EGTA, 2 mM dithiothreitol, 0.01% Brij 35 and 50% glycerol. **Store at –80°C**

Applications: Lambda PP 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: ~800,000 units/mg.

Molecular Weight: 25,000 daltons.

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

Quality Control Assays

Protease Activity: After incubation of 10,000 units of Lambda PP with a standardized mixture of proteins for 2 hours at 30°C, no proteolytic activity could be detected by SDS-PAGE and Coomassie Blue staining.

Heat Inactivation: 65°C for 1 hour in the presence of 50 mM Na_2EDTA

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 Lambda PP.

100 units of Lambda PP remove ~ 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 Serine/threonine Phosphatase (PSP) activity of Lambda PP is assessed on MyBP phosphorylated exclusively on serine/threonine residues with cAMP-dependent Protein Kinase. The Protein Tyrosine Phosphatase (PTP) activity is assessed on MyBP phosphorylated exclusively on tyrosine residues with Abl Protein Tyrosine Kinase.

Lambda PP is active on phosphorylated histidine residues (2).

Lambda PP is inhibited by vanadate (2).

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

(see other side)

CERTIFICATE OF ANALYSIS

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Lambda PP is inhibited by vanadate (2).

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

(see other side)

CERTIFICATE OF ANALYSIS

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 Lambda PP (100 units) are found when the reaction buffer and $MnCl_2$ are supplemented with:

10 mM Sodium Orthovanadate (NEB #B0758) (3)	80%
10 mM Sodium Orthovanadate, 50 mM Sodium Fluoride (NEB #B0759)	90%
50 mM Na_2 EDTA	95%
1% Triton X-100	no
0.4% Nonidet P-40	no
0.025% Tween 20	no
0.5 M NaCl	5%
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. Cohen, P.T.W. and Cohen, P. (1989) *Biochem. J.* 260, 931–934.
2. Zhuo, S. et al. (1993) *J. Biol. Chem.* 268, 17754–17761.
3. 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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