Lambda Protein Phosphatase (Lambda PP)

Supplied in: 100 mM NaCl, 50 mM HEPES (pH 7.5 @ 25°C), 0.1 mM MnCl₂, 0.1 mM EGTA, 2 mM dithiothreitol, 0.01% Brij 35 and 50% glycerol. Store at –70°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 MnCl₂ (10 mM)

Reaction Conditions: 1X NEBuffer for PMP, supplemented with 1 mM MnCl₂.
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 Na₂EDTA

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 Ab1 Protein Tyrosine Kinase.

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

Lambda PP is inhibited by vanadate (2).

(See other side)
Optimal incubation times and enzyme concentrations must be determined empirically for each particular substrate.

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:

Companion Products:
- NEBuffer Pack for Protein MetalloPhosphatases #B0760S
- Sodium Orthovanadate #P0758S
- Sodium Fluoride #P0759S
- p-Nitrophenyl Phosphate (PNPP) #P0757S

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