Lambda Protein Phosphatase (Lambda PP)

**Description:** Lambda Protein Phosphatase (Lambda PP) is a Mn²⁺-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 under the control of a T7 expression system (kindly provided by Dr. D. Barford) (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).

**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 Tyrosine Phosphatase (PTP) activity of Lambda PP is assessed on MyBP phosphorylated exclusively on tyrosine residues with cAMP-dependent Protein Kinase. The Protein Tyrosine Phosphatase (PTP) activity is assessed on MyBP phosphorylated exclusively on tyrosine residues with Abi 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.

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- 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.
- 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.
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 MnCl₂ are supplemented with:

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>[%]</th>
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<tbody>
<tr>
<td>10 mM Sodium Orthovanadate</td>
<td>80%</td>
</tr>
<tr>
<td>10 mM Sodium Orthovanadate, 50 mM Sodium Fluoride</td>
<td>90%</td>
</tr>
<tr>
<td>50 mM Na₂ EDTA</td>
<td>95%</td>
</tr>
<tr>
<td>1% Triton X-100</td>
<td>no</td>
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<tr>
<td>0.4% Nonidet P-40</td>
<td>no</td>
</tr>
<tr>
<td>0.025% Tween 20</td>
<td>no</td>
</tr>
<tr>
<td>0.5 M NaCl</td>
<td>5%</td>
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<tr>
<td>ATP Mix (10 mM MgCl₂, 0.1 mM ATP)</td>
<td>no</td>
</tr>
<tr>
<td>Protease Inhibitor Cocktail*</td>
<td>10%</td>
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</tbody>
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*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