

## Lambda Protein Phosphatase (RNase-free)

### Introduction

Lambda Protein Phosphatase (Lambda PP) is a  $Mn^{2+}$  dependent protein phosphatase exhibiting dephosphorylation activity toward phosphorylated serine, threonine, and tyrosine residues. It is a protein consisting of 221 amino acids encoded by the open reading frame ORF221 of lambda phage. Lambda PP can be used to remove phosphate groups from proteins, facilitating research on the relationship between protein phosphorylation and its activity or structure, as well as validating the specificity of phospho-site-specific antibodies.

### Components and Storage

Size	20 KU	100 KU	200 KU	Storage
Components				
Lambda PP (RNase-free)	0.2 mL	1 mL	2 mL	-80°C
10X Lambda PP Reaction Buffer	1 mL	5 x 1 mL	10 x 1 mL	-80°C
10X MnCl <sub>2</sub> (10mM MnCl <sub>2</sub> )	1 mL	5 x 1 mL	10 x 1 mL	-80°C
Shipping: dry ice		Shelf life: 2 years		

**Note:** It is recommended to aliquot upon first use to avoid repeated freeze-thaw cycles: Store aliquots at -20°C for a validity period of 6 months.

### Protocol

The optimal incubation time and enzyme concentration must be determined empirically for each specific substrate. For example, in a 50  $\mu$ L reaction, 100 U Lambda PP can remove about 100% phosphate (0.25 nmol) in the phosphorylated myelin alkaline proteins (phospho-MyBP, 18.5 kDa) within 30 minutes.

- Set up the reaction system in the ice bath as follows:

Reagent	Volume	Final Concentration
Protein	X $\mu$ L	5 $\mu$ M
10X Lambda PP Reaction Buffer	5 $\mu$ L	1X
10X MnCl <sub>2</sub>	5 $\mu$ L	1 mM
Lambda PP	1 $\mu$ L	-
Nuclease-free Water	To 50 $\mu$ L	

- Mix and incubate at 30°C for 30 min.

## Properties

1. Definition of Enzyme Units: In a 50  $\mu$ L reaction system, one unit will hydrolyze 1 nmole of p-nitrophenyl phosphate (50 mM) per min at pH 7.5 at 30°C.
2. Ingredients of Lambda PP solution: 50 mM HEPES (pH 7.5, 25°C), 100 mM NaCl, 2 mM DTT, 0.01% BRIJ 35, 0.1mM EGTA, 0.1 mM  $MnCl_2$ , 50% Glycerol.
3. Ingredients of 10X Lambda PP Reaction Buffer: 500 mM HEPES (pH 7.5, 25°C), 1 M NaCl, 20 mM DTT, 0.1% BRIJ 35.
4. Quality assurance:
  - a) Protein purity > 95%.
  - b) No RNase contamination, no protease, nuclease enzyme and exonuclease contamination.
5. For research use only. Not for human, veterinary diagnostics or therapeutic use.

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