

Lambda Protein Phosphatase

02-300 20,000 U $(400\text{U}/\mu\text{l})$ 02-300-5 $5 \times 20,000 \text{ U}$ $(400\text{U}/\mu\text{l})$

Storage: Store at -80°C. Avoid freeze-thaw cycles.

Product: Functional full-length recombinant λ Protein Phosphatase (λ -PPase) expressed in E.coli. It is a Mn+2-dependent protein phosphatase with activity towards phosphorylated serine, threonine, tyrosine and histidine residues. It is the 221 amino-acid product of ORF221 open reading frame on bacteriophage lambda (1, 2). This product is an intact enzyme of high quality without tag.

Applications: λ -PPase can be used to release phosphate groups from phosphorylated serine, threonine, tyrosine and histidine residues in proteins (2). It should be noted that different proteins are dephosphorylated at different rates. Optimal reaction temperature is 30°C. Inclusion of protease inhibitor cocktail and shortest incubation time is desired when assays are done with crude samples.

Form: 400 U/ul λ-PPase in 50mM HEPES (pH 7.5), 100mM NaCl, 2mM dithiothreitol, 0.1 mM MnCl₂, 0.1mM EDTA, 50% glycerol, 0.01% Brij 35.

Activity: 400 U/ul, where one unit is defined as the amount of enzyme that hydrolyzes 1 nmole of p-nitrophenyl phosphate per minute at 30° C. Unit definition assays are performed with 50mM p-nitrophenyl phosphate in λ -PPase buffer, supplemented with 2 mM MnCl₂ in a 50 ul reaction.

Specific Activity: ~400,000 U/mg

Purity: Greater than 95% as determined by SDS-PAGE (CBB staining) that contains no detectable protease activity

Reagents Supplied with Enzyme:

(1)10 x λ-PPase Reaction Buffer: [500mM Tris-HCl (pH 7.6), 1M NaCl, 20mM dithiothreitol, 1mM EDTA, 0.1% Brij 35]
(02-Lpb 1ml)
(2)10 x Mn²⁺ (20 mM MnCl₂)
(02-Lmn 1ml)

Data Link: UniProtKB/Swiss-Prot P03772 (PP_LAMBD)

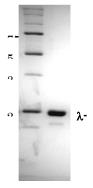


Fig.1 SDS-PAGE of λ -Protein Phosphatase

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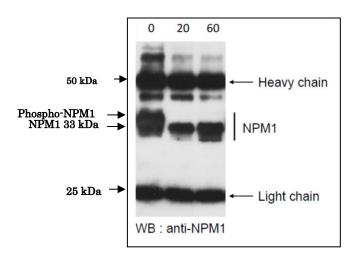


Fig.2 Dephosphorylation of phospho - NPM1 protein by incubation with λprotein phosphatase in vitro.

HeLa cells treated with Nocodazole was lysed, and NPM1 protein was immunoprecipited with anti-NPM1 antibody. The precipitate was suspended in 50 ul of λ protein phosphatase reaction buffer added with 5 ul of the protein phosphatase and incubated at 30 °C for the indicated time (min). The reaction products were analyzed by western blotting.

Reference: This product has been used in the following publications.

- 1.Tojima T et al. Steering neuronal growth cones by shifting the imbalance between exocytosis and endocytosis. J Neurosci. 2014 May 21;34(21):7165-78. PMID: 24849351
- 2.Moriyama T et al.SUMO-modification and elimination of the active DNA demethylation enzyme TDG in cultured human cells. <u>Biochem Biophys Res Commun.</u> 2014 May 9;447(3):419-24. PMID: <u>24727457</u>
- 3. Yang CC et al. Claspin recruits Cdc7 kinase for initiation of DNA replication in human cells. Nature Commun. 2016, 7: 12135. PMID: 27401717
- Tomii S et al. Cortical Actin Alteration at the Matrix-Side Cytoplasm in Lung Adenocarcinoma Cells and Its Significance in Invasion. <u>Pathobiology.</u>2017; 84(4):171-183. PMID: <u>28002815</u>
- 5.Honda T & Inui M. PDZRN3 regulates differentiation of myoblasts into myotubes through transcriptional and posttranslational control of Id2. J. Cell Physiol. 2019, 234: 2963-2972. PMID: 30066954

Useful References:

- Cohen PTW & Cohen P (1989) "Discovery of a protein phosphatase activity encoded in the genome of bacteriophage λ." *Biochem J.* 260: 931-934 PMID: 2548489
- Zhuo S et al (1993) "Expression, purification, crystallization, and biochemical characterization of recombinant protein phosphatase." J. Biol. Chem. 268:17754-17761 PMID: 8394350

Please note: All products are FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR MILITARY USE.