Lambda Protein Phosphatase

02-300 20,000 U (400U/ul), 02-300-5 5 x 20,000 U (400U/ul)

λ Protein Phosphatase (λ -PPase) 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). **λ -PPase** was expressed as a recombinant protein in *E.coli* and highly purified (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℃. 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 $MnCl_2$, 0.1mM EDTA, 50% glycerol, 0.01% Brij 35.

Shipping and Storage : Sent with ice-pack or dry ice and upon arrival, aliquot and store at -80℃. Avoid repeating freeze-thaw cycles.

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℃. Unit definition assays are performed with 50mM p-nitrophenyl phosphate in λ -PPase buffer, supplemented with 2 mM $MnCl_2$ in a 50 ul reaction.

Specific Activity: 400,000 U/mg

Quality Assurance: Greater than 95% homogeneous protein determined by SDS-PAGE (CBB staining) that contains no detectable protease activity

Reagents Supplied with Enzyme:

10 x λ -PPase Reaction Buffer [500mM Tris-HCl (pH 7.6), 1M NaCl, 20mM dithiothreitol, 1mM EDTA, 0.1% Brij 35]

10 x Mn^{2+} (20 mM $MnCl_2$)

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

References:

1. 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
2. Zhuo S *et al* (1993) "Expression, purification, crystallization, and biochemical characterization of recombinant protein phosphatase." *J. Biol.Chem.***268**:17754-17761 PMID: 8394350

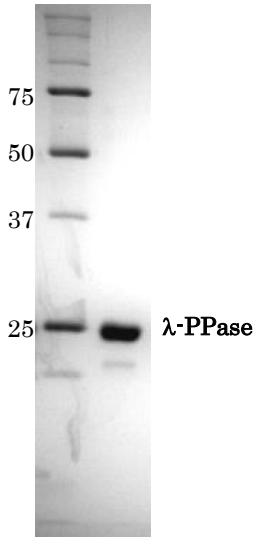

Fig.1 SDS-PAGE of λ -PPase

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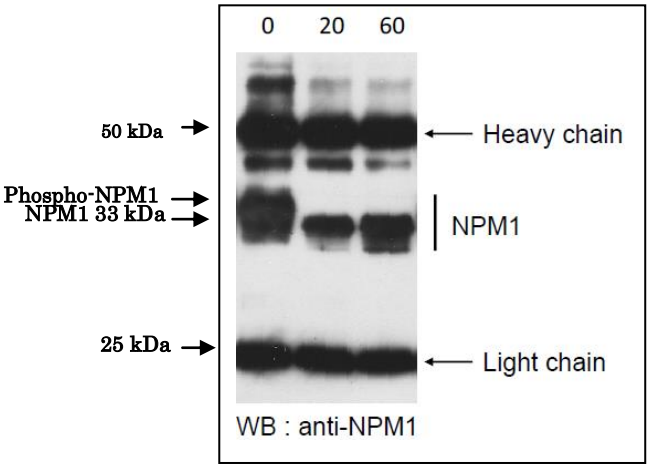

Fig.2 Dephosphorylation of phospho-NPM1 protein by incubation with λ protein phosphatase in vitro.

NPM1 protein in HeLa cells treated with Nocodazole was lyzed, and 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℃ for the indicated time (min). The reaction products were analyzed by western blotting.

Data were kindly provided by Prof. T. Urano at Shimane Univ. School of Medicine.