Lambda Protein Phosphatase

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

Shipping and Storage : Sent at 4℃ or -20℃ and store at -20℃. Do not freeze below -20℃

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℃. 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.

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

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 Mn^{2+} (20 mM $MnCl_2$)　(02-Lmn　1ml)

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

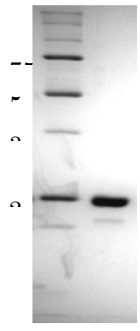

Fig.1　SDS-PAGE　of λ-Protein Phosphatase

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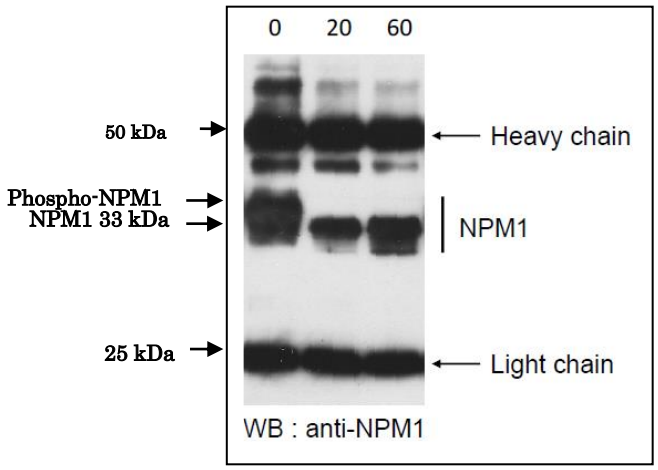

Fig.2 Dephosphorylation of phospho -NPM1 protein by incubation with λprotein phosphatase in vitro.

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℃ for the indicated time (min). The reaction products were analyzed by western blotting.

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

1. Tomii S et al. Cortical Actin Alteration at the Matrix-Side Cytoplasm in Lung Adenocarcinoma Cells and Its Significance in Invasion. Pathobiology.2017; 84(4):171-183. PMID: 28002815
2. 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
3. Moriyama T et al.SUMO-modification and elimination of the active DNA demethylation enzyme TDG in cultured human cells. Biochem Biophys Res Commun. 2014 May 9;447(3):419-24. PMID: PMID: 24727457

Useful 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