Invitrogen
life technologies

Bacterial Alkaline Phosphatase

Cat. No. 18011-015 **Size: 2,500 units**
Conc.: 150 U/µl **Store at -20°C (not frost-free).**

Description:
Bacterial Alkaline Phosphatase (BAP) is a phosphomonoesterase that hydrolyzes 3′ and 5′ phosphates from DNA and RNA. It is suitable for removing 5′ phosphates prior to end-labeling and for dephosphorylating vectors prior to insert ligation. BAP is active at 65°C for at least 1 h. It is sensitive to inhibition by micromolar amounts of inorganic phosphate. The enzyme is purified from *E. coli* C90.

Components:

18011-015	BAP
Y02290	10X Dephosphorylation Buffer

Unit Definition:
One unit hydrolyzes 1 nmol of ATP in 30 min at 37°C.

Storage Buffer:	10X Dephosphorylation Buffer:
10 mM Tris-HCl (pH 8.0)	100 mM Tris-HCl (pH 8.0)
120 mM NaCl	
50% (v/v) glycerol	Store buffer at 4°C or -20°C.

Quality Control:
This product has passed the following quality control assays: absence of detectable endodeoxyribonuclease, exodeoxyribonuclease and ribonuclease activities; performance in dephosphorylating blunt-ended DNA. The enclosed buffers were assayed with the enzyme and met quality control specifications.

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Traditional Protocol:

This protocol dephosphorylates 1 pmol of 5′-DNA termini from purified DNA. DNA dephosphorylated by this method is suitable for cloning or for labeling by T4 polynucleotide kinase using the Forward Reaction:

1. Determine the mass of DNA required for 1 pmol of the type of DNA 5′ end.
2. To a 1.5-ml microcentrifuge tube, add 4 µl of 10X Dephosphorylation Buffer and 1 pmol of DNA ends.
3. Add autoclaved, distilled water to 39 µl.
4. Dilute BAP in dilution buffer such that 1 µl contains 70 units of enzyme.
5. Incubate the reaction at 65°C for 1 h.
6. Inactivate/remove the BAP according to the protocol described below.

Simplified Protocol:

This protocol allows for the dephoshporylation of DNA directly in restriction endonuclease buffer in the presence of the restriction endonuclease. This is a convenient way of preparing DNA for cloning.

1. Restriction endonuclease digest the vector DNA. (**NOTE:** Heat inactivation of the restriction endonuclease and subsequent purification of the vector DNA are not necessary.)
2. Add 1 µl of BAP (150 units) to the restriction endonuclease digest.
3. Incubate the reaction at 65°C for 1 h.
4. Inactivate/remove the BAP according to the protocol described below.

Inactivation/Removal of Bacterial Alkaline Phosphatase (Organic Extraction):

Add an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1). Vortex thoroughly and centrifuge at 14,000 × *g* at room temperature for 5 min. Carefully remove the upper, aqueous phase and transfer it to a fresh microcentrifuge tube. Add 0.1 volume of 3 M sodium acetate. Vortex. Add 2.5 volumes of 100% EtOH. (**NOTE:** Do not substitute NH_4OAc for NaOAc because NH_4 ions inhibit T4 polynucleotide kinase.) Vortex the mixture thoroughly and centrifuge at 14,000 × *g* at room temperature for 5 min.