For research use only

ISO9001

Bacterial Alkaline Phosphatase

Product	Quantity	Cat. No.	Remarks
Bacterial Alkaline Phosphatase	100 unit	EBT-3032	0.5 unit/㎕

Description

Bacterial Alkaline Phosphatase catalyzes the hydrolysis of 5´-phosphate groups from DNA, RNA, and ribo- and deoxyribonucleoside triphosphates. This enzyme is used to prevent recircularization and religation of linearized cloning vector DNA by removing phosphate groups from both 5´-termini and may also be used for the dephosphorylation of 5´ phosphorylated ends of DNA or RNA for subsequent labeling with [γ-^{32}P]ATP and T4 Polynucleotide Kinase. Bacterial Alkaline Phosphatase is purified from recombinant *E.coli*.

Concentration & Storage Condition

0.5 unit/μl. Store at -20℃.

Storage Buffer

50 mM Tris-HCl, pH 8.0, 100 mM KCl, 1 mM $MgSO_4$, 50% glycerol.

10x Reaction Buffer

500 mM Tris-HCl, pH 9.0, 10 mM $MgCl_2$.

Unit Definition

One unit is defined as the amount of enzyme required to catalyze the hydrolysis of 1 μmol of 4-nitrophenyl phosphate per min at 37°C in 1 M diethanolamine, 10.9 mM 4-nitrophenyl phosphate, 0.5 mM $MgCl_2$, pH 9.8.

QC Tests

Activity, exo and endonuclease activity test, SDS-PAGE purity, performance tests.

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Usage Information

1. Protocol for Dephosphorylation of DNA 5'-termini

1) Add the following components to the microcentrifuge tube :

DNA solution	10-40 μl (1-20 pmol termini)
10x Reaction Buffer	5 μl
Bacterial Alkaline Phosphatase	0.5 μl
Nuclease-Free Water to final volume	50 μl

2) Incubate at 37°C for 60 min.
3) Add Proteinase K to a final concentration of 1ug/ml, then incubate at 37°C for 30 min, extract DNA with phenol/chloroform and precipitate with ethanol.

2. 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 Bacterial Alkaline Phosphatase to the restriction endonuclease digest.
3) Incubate the reaction at 37°C for 1 hour.
4) Add Proteinase K to a final concentration of 1mg/ml, then incubate at 37°C for 30 min, extract DNA with phenol/chloroform and precipitate with ethanol.

* Phenol extraction and Ethanol precipitation procedure

1) 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.
2) Carefully remove the upper, aqueous phase and transfer it to a fresh microcentrifuge tube.
3) Add 0.1 volume of 3 M sodium acetate. Mix
4) Add 2.5 volumes of 100% EtOH. Vortex the mixture thoroughly
   (NOTE**:** Do not substitute NH_4OAc for NaOAc because NH_4 ions inhibit T4 polynucleotide kinase.)
5) Centrifuge at 14,000 × *g* at room temperature for 5 min.
6) Dry and dissolved in TE buffer