Alkaline Phosphatase

Cat.No.: HYJ238

Product Description

Alkaline Phosphatase is derived from a recombinant *E. coli* strain that carries the TAB5 gene. The enzyme catalyzes the dephosphorylation of 5′ and 3′ ends of DNA and RNA phosphomonoesters. Also, it hydrolyses ribose, as well as deoxyribonucleoside triphosphates (NTPs and dNTPs). TAB5 Alkaline Phosphatase acts on 5′ protruding, 5′ recessed and blunt ends. The Phosphatase can be used in many molecular biology applications, such as cloning or probe end labeling to remove the phosphorylated ends of DNA or RNA. In cloning experiments, dephosphorylation prevents the linearized plasmid DNA from self-ligation. It can also degrade unincorporated dNTPs in PCR reactions to prepare a template for DNA sequencing. The enzyme is completely and irreversibly inactivated by heating at 70°C for 5 minutes, thereby making removal of the phosphatase prior to ligation or end labeling unnecessary.

Components

Components	0.5 KU	2.5 KU	50 KU
Alkaline Phosphatase	0.1 mL	0.5 mL	10 mL

Storage

Store at -25 ~ -15 ℃, valid for 2 years (Avoid repeated freeze-thaw cycles)

Storage Buffer

10 mM Tris-HCl (pH 7.4, 25℃), 1 mM $MgCl_2$, 0.01 mM $ZnCl_2$, 50% glycerol.

Unit Definition

One unit is defined as the amount of enzyme that will dephosphorylate 1 μg of pUC19 vector DNA cut with HindIII (5′protruding ends), HincII (blunts ends) or PstI (5′recessed ends) in 30 minutes at 37°C. Dephosphorylation is defined as > 95% inhibition of recirculation in a self-ligation reaction and is measured by transformation into *E. coli*.

Molecular Weight

The molecular weight of Alkaline Phosphatase is 35 kDa.

Heat Inactivation

70℃ for 5 minutes

Quality Control

1. **Exonuclease:** 5 U of Alkaline Phosphatase with 1 μg λ-Hind III digest DNA at 37 ℃ for 16 hours yields no degradation as determined by agarose gel electrophoresis.
2. **Endonuclease:** 5 U of Alkaline Phosphatase with 1 μg λDNA at 37 ℃ for 16 hours yields no degradation as determined by agarose gel electrophoresis.
3. **Nickase:** 5 U of Alkaline Phosphatase with 1 μg pBR322 at 37 ℃ for 16 hours yields no degradation as determined by agarose gel electrophoresis.
4. **RNase:** 5 U of Alkaline Phosphatase with 1.6 μg MS2 RNA for 16 hours at 37 ℃ yields no degradation as determined by agarose gel electrophoresis.
5. ***E. coli* DNA:** 5 U of Alkaline Phosphatase is screened for the presence of *E. coli* genomic DNA using TaqMan qPCR with primers specific for the *E. coli* 16S rRNA locus. The *E. coli* genomic DNA contamination is ≤0.1 pg/5 U.
6. **Bacterial Endotoxin:** LAL-test, according to Chinese Pharmacopoeia IV 2020 edition, gel limit test method, general rule (1143). Bacterial endotoxin content should be ≤10 EU/mg.

Reaction system and conditions

1. Add the following components in the order specified (Protocol for dephosphorylation of 5′-ends of DNA)

Components	Dosage
DNA	1 pmol of DNA ends*
10× Reaction Buffer**	2 μL
TAB5 Alkaline Phosphatase	5 U
ddH_2O	Up to 20 μL

* 1 pmol of DNA ends is about 1 μg of a 3 kb plasmid.

**10× Reaction Buffer: 500 mM Bis-Tris-Propane HCl（pH 6.0，25°C），10 mM $MgCl_2$，1 mM $ZnCl_2$

2. Incubate at 37°C for 30 minutes.
3. Stop reaction by heat-inactivation at 70°C for 5 minutes.

Applications

1. Dephosphorylation of DNA and RNA
2. Prevention of recircularization of cloning vectors
3. Removal of dNTPs and pyrophosphate from PCR reactions
4. Preparation of templates for 5′ end labeling

Notes

1. The reaction system can be scaled up or down according to the experimental demands.
2. The enzyme is homologous dimer, and its monomer molecular weight is 35 kDa.
3. It is indispensable to involve Zn^{2+} in the reaction process since it is a Zn^{2+} or Mg^{2+} dependent enzyme.
4. For your safety, please wear lab coat and disposable gloves during operation.