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Thermolabile alkaline phosphatase

Cat. Number: E-12005, E-12050

Enzyme description

This product is a recombinant enzyme - alkaline phosphatase of the gram-negative bacterium *Vibrio splendidus*. The enzyme has a molecular weight of ~58 kDa and exhibits catalytic activity in its homodimeric form [1]. The temperature range of the enzyme is from 15 to 37°C, but for prolonged reactions it is recommended to use 15°C as the most optimal for enzyme stability [2]. Thermolabile alkaline phosphatase removes phosphates from the 5' and 3' ends of DNA and RNA and can be used instead of Antarctic phosphatase.

Application

- Cloning of restriction fragments.
- mRNA synthesis.

Source

Thermolabile alkaline phosphatase is isolated from an *E. coli* strain containing a plasmid with a cloned *Vibrio splendidus* enzyme gene.

Activity Units

One unit of activity hydrolyzes 1 μmol of para-nitrophenylphosphate (pNPP) in 0.5 ml of the reaction mixture in 15 min at 25°C, in a standard reaction buffer (10 mM Tris-HCl (pH 9.0 at 25°C); 10 mM $MgCl_2$; 100 mM NaCl; 1 mM DTT, 10 mM pNPP).

Enzyme concentration and packaging: 5000 U/ml.

Cat. No.	Product Name	Quantity	Volume
E-12005	Thermolabile alkaline phosphatase	500 U	100 μl
E-12050		5000 U	1000 μl

Storage Buffer

25 mM Tris (pH 8.0 at 25°C), 2 mM $MgCl_2$, 0.02 mM $ZnCl_2$, 50% glycerol

Quality Control
Each batch of enzyme is tested for enzyme activity, electrophoretic purity in SDS-PAGE, and nonspecific proteolytic activity.

10x Standard reaction buffer
100 mM Tris-HCl (pH 9.0 at 25°C), 100 mM $MgCl_2$, 1 M NaCl, 10 mM DTT (1 ml 10x buffer supplied with enzyme).

Typical protocol for dephosphorylation of hydrolyzed plasmids
1. Mix the following components in a test tube:
   - 2 µl of 10x standard reaction buffer;
   - 1 µg of hydrolyzed plasmid DNA (4-8 kb);
   - up to 19 µl of nuclease-free water;
   - 1 µl (5 U) thermolabile alkaline phosphatase.
2. Incubate the reaction mixture at 37°C for one hour.
3. To inactivate the enzyme, heat the reaction mixture at 65°C for 20 minutes.
4. The processed plasmid DNA can be used in further work. If necessary, DNA can be further purified using well-known methods, for example, a Kit for isolating DNA and RNA from reaction mixtures (cat. no. DR-10, DR-50, DR-250).

Enzyme inactivation
Incubation for 20 minutes at 65°C.

Storage and transportation conditions
Store at -20°C.
Transportation is allowed at a temperature not exceeding +8°C for two days.

References

1. Helland, R., Larsen, R. L., & Ásgeirsson, B. (2009). The 1.4 Å crystal structure of the large and cold-active Vibrio sp. alkaline phosphatase. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics*, *1794*(2), 297-308.
2. Hauksson, J. B., Andrésson, Ó. S., & Ásgeirsson, B. (2000). Heat-labile bacterial alkaline phosphatase from a marine Vibrio sp. *Enzyme and microbial technology*, *27*(1-2), 66-73.