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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.