



Heat Sensitive Phosphatase

Product Number: DB003

Shipping and Storage

-20°C.

Components

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Heat Sensitive Phosphatase (5U/μL)	40μL
10×HSP Reaction Buffer	1mL

Description

This enzyme is obtained by expressing the phosphatase gene isolated from the Antarctic cold loving strain TAB5 in Escherichia coli and purifying it multiple times. Phosphatases can catalyze the removal of 5 'phosphate groups at the ends of DNA or RNA. After treating the vector DNA with thermosensitive phosphatase during cloning, its fragment lacks the necessary 5 'phosphate terminus for ligase, so the vector cannot perform self ligation, which can greatly reduce the background of vector self ligation. This enzyme can act on the 5 'protruding end, concave end, and flat end.

Application

1. Remove the phosphate group at the 5 'end of the carrier to reduce the background of self connection;
2. Remove residual dNTP and pyrophosphate from PCR products;
3. Remove the phosphate group at the 5 'end of the DNA probe and prepare the optimal substrate for phosphorylation labeling at the 5' end;
4. Protein dephosphorylation.

Usage recommendations

Thermally sensitive phosphatase exhibits optimal enzyme activity in a 1×HSP buffer. If the reaction is to be carried out in a restriction enzyme buffer, 10×HSP buffer must be added to the reaction system to a final concentration of 1×and incubated at 37°C.

Quality assurance

After multiple column purifications, SDS-PAGE gel detection showed only a clear and single target band; The PCR method detects no residual E. coli DNA and no contamination of nucleic acid endonucleases.

Definition of Activity

The amount of enzyme required to dephosphorylate 1μg of pUC19 DNA digested with Hind III (producing a 5 ' protruding end), EcoR V (producing a smooth end), or Pst I (producing a 5 ' concave end) at 37°C for 30 minutes is defined as one unit.

The dephosphorylation standard refers to the ability to inhibit>95% of DNA fragments from rewiring in the ligation reaction (detected by electrophoresis).

Reaction conditions

1 × HSP Reaction Buffer [50mM Bis Tris propane HCl, 1mM MgCl₂, 0.1mM ZnCl₂ (pH 6.0 at 25°C)], incubated at 37°C.

Thermal deactivation

70°C, 5 minutes.