Polar-BAP

Thermosensitive Bacterial Alkaline Phosphatase

Cat. No.	size
E1027-01	1000 u
E1027-02	5000 u

Thermosensitive Bacterial Alkaline Phosphatase catalyzes the release of 5'- and 3'-phosphate groups from DNA, RNA, and nucleotides.

Description:

- Removes 5'-phosphates from DNA, RNA, rNTPs and dNTPs.
- Heat inactivation in 5 minutes at 70°C.
- Resistant to chemical changes and active over a broad range of buffer conditions.
- Degradation of dNTPs prior to sequencing of PCR product.
- Can be used to remove 5'-phosphates from DNA or RNA prior to 5'-end labeling (1).
- Removes 5'-phosphates from linearized vector molecules to prevent self-ligation of the vector during cloning procedures (1).
- Protein dephosphorylation.
- Ideal for diagnostic immunoassays and immunodetection of proteins and nucleic acids following blotting experiments (1).

Unit Definition:

One unit is the amount of enzyme required to dephosphorylate 1 µg of linearized pUC19 vector DNA in 30 min at 37°C.

Storage Conditions:

Store at -20°C.

Inactivation Temperature (5 min): 70°C

Quality Control:

All preparations are assayed for contaminating endonuclease and nonspecific RNase and single- and double-stranded DNase activities.

References:

1. *Sambrook (1989) Molecular Cloning: A Laboratory Manual, (2nd ed.). 5.72.*
2. *Werle et al. (1994) Nuc. Acids Research 22(20): 4354–4355.*

Standard PCR Clean-up Protocol:

Mix the following reaction components:

25-50 µl of PCR just after amplification

0.5 µl of Exonuclease I 20 U/µl (Cat. No. E1150)

1 µl of Polar-BAP 5 U/µl

Incubate for 15 min at 37°C.

Heat Inactivate 15 min 80°C.

- Up to 5 µl may be used directly to sequencing without any other purification.
- It is recommended to use PCR without unspecific products.
- No specific buffer required.

Standard Vector Dephosphorylation Protocol:

Mix the following reaction components:

1-5 µg of DNA cut with any restriction enzyme

5 µl 10 x Polar-BAP Reaction Buffer

1 µl of Polar-BAP 5 U/µl

Nuclease-free water: to 50 µl

Incubate for 30 min at 37°C.

Heat Inactivate 5 min 70°C.

1 x Polar-BAP Reaction Buffer:

50 mM Bis-Tris-Propane-HCl (pH 6.0 at 25°C), 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$.

Storage Buffer:

10 mM Tris-HCl (pH 7.6 at 22°C), 1 mM $MgCl_2$, 0.01 mM $ZnCl_2$, 1 mM dithiothreitol, 50% glycerol.

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