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roboklon

Polar BAP

Thermosensitive Bacterial Alkaline Phosphatase

Polar Bacterial Alkaline Phosphatase

Cat. No.	Size
E1027-01	1.000 units
E1027-02	5.000 units

Unit Definition:
One unit is the amount of enzyme required for dephosphorylation of 1 µg of linearized pUC19 vector DNA in 30 min at 37˚C.

Storage Conditions:
Store at -20˚C

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).

Standard Exo I PCR Clean-up Protocol (for subsequent sequencing):

Mix the following reaction components:
25-50 µl of PCR just after amplification
0.5 µl 10 U Exonuclease I (Cat. No. E1150)
1 µl 5 U Polar-BAP
Incubate for 15 min at 37˚C
Heat inactivation for 15 min at 80˚C
Up to 5 µl may be used directly to sequencing without any further purification
it is recommended to use PCR devoid of any non-specific products
Known to work in a variety of different buffer systems. No dedicated buffers are 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 5 U Polar-BAP
@ 50 µl H_2O, nuclease free
Incubate for 30 min at 37˚C
Heat inactivation for 5 min at 70˚C

1 x Polar-BAP Reaction Buffer:

50 mM Bis-Tris-Propane-HCl pH 6 @ 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 DTT, 50% glycerol.

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*

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