Antarctic Phosphatase

Cat. No. : RK20537

Product components

Components	Component number	Concentration	Size-1 1,000 U	Size-2 5,000 U
Antarctic Phosphatase	RM21308	5,000 U/mL	200 μL	1 mL
10X Antarctic Phosphatase Reaction Buffer	RM20152	10X	1.25 mL	2 × 1.25 mL

Product Description

Antarctic Phosphatase (AnP) is a heat-labile alkaline phosphatase purified from a recombinant source. AnP nonspecifically catalyzes the dephosphorylation of 5 ′ and 3 ′ ends of DNA and RNA phosphomonoesters. Also, AnP Hydrolyses ribo-, as well as deoxyribonucleoside triphosphates (NTPs and dNTPs). AnP is useful in many molecular biology applications such as the removal of phosphorylated ends of DNA and RNA for subsequent use in cloning or end-labeling of probes. In cloning, dephosphorylation prevents religation of linearized plasmid DNA. The enzyme acts on 5’ protruding, 5’recessed, and blunt ends. AnP may also be used to degrade unincorporated dNTPs in PCR reactions to prepare templates for DNA sequencing or SNP analysis. AnP is completely and irreversibly inactivated by heating at 80°C for 2 minutes, thereby making removal of AnP prior to ligation or end-labeling unnecessary.

Product Source

Antarctic Phosphatase was expressed and purified from *E. coli*.

Storage

Store at -20℃ and avoid repeated freeze-thaw cycles

Unit Definition

One unit is defined as the amount of enzyme that will dephosphorylate 1 μg of pUC19 vector DNA cut with a restriction enzyme generating 5’ recessed ends in 30 minutes at 37°C.

Reaction Conditions

1X Antarctic Phosphatase Reaction Buffer, incubate at 37°C

1X Antarctic Phosphatase Reaction Buffer

50 mM Bis-Tris-Propane-HCl, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, pH 6.0 @ 25°C

Storage Conditions

10 mM Tris-HCl, 1 mM $MgCl_2$, 0.01 mM $ZnCl_2$, 50 % Glycerol, pH 7.4 @ 25°C

Heat Inactivation

80°C for 2 min.

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Operation Description

1. Set-up the following reaction on ice, noting that the enzyme should be added at the end and then gently mix the reaction by pipetting up and down and microcentrifugation.

Components	20 μL Reaction
10X Antatctic Phosphatase Rection Buffer	2 μL(1X)
Substrate	to 1 pmol DNA
Antatctic Phosphatase	1 μL(5 units)
Nuclease-Free-Water	to 20 μL

2. Incubate at 37℃ for 30 minutes.
3. Terminate the reaction by heat inactivation at 80℃ for 2 min.

Dephosphorylation of 5'-ends of DNA in Restriction Enzyme Reaction

1. The phosphatase can be added directly into the digestion reaction during or after DNA digestion.
2. Antarctic Phosphatase is active in ABuffer A/B/C/S and CutA/B/C/S buffers only when supplemented with Antarctic Phosphatase Reaction Buffer, which provides Zn2+ required for enzyme activity.
3. The restriction enzyme should be heat inactivated at the same time as the phosphatase after digest and dephosphorylation. If restriction enzyme cannot be heat inactivated, DNA purification is required before ligation.

Notes

1. Adding 1/10 volume of 10X Antarctic Phosphatase Reaction Buffer will provide the amount of Zn^{2+} that the enzyme requires for activity.
2. Antarctic Phosphatase is also active in ABuffer A/B/C/S and Cut A/B/C/S buffers only when supplemented with 1/10 volume of the 10X Antarctic Phosphatase Reaction Buffer.
3. Antarctic Phosphatase activity is enhanced in the presence of monovalent salts.
4. Antarctic Phosphatase is inhibited by metal chelators (e.g. EDTA), inorganic phosphate and phosphate analogs. Antarctic Phosphatase activity is decreased in the presence of reducing agents (DTT, β-ME).
5. Antarctic Phosphatase, as are most alkaline phosphatases, is a Zn^{2+} and Mg^{2+} -dependent enzyme and does require supplemental zinc.

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