Antarctic Phosphatase

1-800-632-7799
info@neb.com
www.neb.com

M0289S 022150417041

M0289S

1,000 units **5,000 U/ml** **Lot: 0221504**

RECOMBINANT **Store at –20°C** **Exp: 4/17**

Description: Antarctic Phosphatase catalyzes the dephosphorylation of 5´ and 3´ ends of DNA and RNA phosphomonoesters. Antarctic Phosphotase also hydrolyses ribo-, as well as deoxyribonucleoside triphosphates (NTPs and dNTPs). Antarctic Phosphatase can be used 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. Antarctic Phosphatase may also be used to degrade unincorporated dNTPs in PCR reactions to prepare templates for DNA sequencing. The enzyme is completely and irreversibly inactivated by heating at 70°C for 5 minutes, thereby making removal of Antarctic Phosphatase prior to ligation or end-labeling unnecessary.

Heat Inactivated In 5 Minutes at 70°C

Source: An *E. coli* strain that carries the TAB5 AP gene, originally cloned in plasmid pNI, recloned in plasmid pEGTAB7–4.1(1,2).

Applications:

- Dephosphorylation of DNA and RNA
- Prevention of recircularization of cloning vectors
- Preparation of templates for 5´ end labeling
- Removal of dNTPs and pyrophosphate from PCR reactions

Supplied in: 10 mM Tris-HCl (pH 7.4), 1 mM $MgCl_2$, 0.01 mM $ZnCl_2$ and 50% glycerol.

Reagents Supplied with Enzyme:
10X Antarctic Phosphatase Reaction Buffer.

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

Unit Definition: One unit is defined as the amount of enzyme that will dephosphorylate 1 µg of pUC19 vector DNA cut with HindIII (5´ protruding ends), HincII (blunts ends) or PstI (5´ recessed ends) in 30 minutes at 37°C. Dephosphorylation is defined as > 95% inhibition of recirculation in a self-ligation reaction and is measured by transformation into *E. coli.*

Unit Assay Conditions: Vector DNA is dephosphorylated in restriction endonuclease buffer supplemented with Antarctic Phosphatase Reaction Buffer. Ligation is performed with 50 ng of vector using the NEB Quick Ligation Kit (NEB #M2200).

Heat Inactivation: 70°C for 5 minutes.

Protocol for Dephosphorylation of 5´-ends of DNA using Antarctic Phosphatase

1. Prepare a 20 µl reaction as follows:

DNA	1 pmol of DNA ends*
Antarctic Phosphatase Reaction Buffer (10X)	2 µl
Antarctic Phosphatase	5 units
H_2O, purified	to 20 µl**

2. Incubate at 37°C for 30 minutes.
3. Stop reaction by heat-inactivation at 70°C for 5 minutes.

* *Note: 1 pmol of DNA ends is about 1 µg of a 3 kb plasmid.*

** *Scale larger reaction volumes proportionally.*

(see other side)

CERTIFICATE OF ANALYSIS

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Antarctic Phosphatase	5 units
H_2O, purified	to 20 µl**

2. Incubate at 37°C for 30 minutes.
3. Stop reaction by heat-inactivation at 70°C for 5 minutes.

* *Note: 1 pmol of DNA ends is about 1 µg of a 3 kb plasmid.*

** *Scale larger reaction volumes proportionally.*

(see other side)

CERTIFICATE OF ANALYSIS

Protocol for Dephosphorylation of 5′-ends of DNA using Antarctic Phosphatase in Restriction Enzyme Reaction

1. Digest 1–5 µg of plasmid DNA in a 20 µl reaction as follows:

DNA	≥ 1 µl
Restriction Enzyme Buffer (10X)	2 µl
Restriction Endonuclease	1 µl
H_2O, purified	to 20 µl

Note: Scale larger reaction volumes proportionally.

2. Incubate at 37°C for 60 minutes or follow manufacturer's recommendations.
3. Add 1/10 volume of 10X Antarctic Phosphatase Reaction Buffer and 5 units of Antarctic Phosphatase for every 1 pmol of DNA ends (about 1 µg of a 3 kb plasmid) and incubate at 37°C for 30 minutes.
4. Stop reaction by heat-inactivation of Antarctic Phosphatase and restriction enzyme (follow manufacturer's recommendations).

Note: If restriction enzyme cannot be heat-inactivated, DNA purification is required before ligation.

Quality Control Assays

Exonuclease Activity: Incubation of a 50 µl reaction containing 50 units of Antarctic Phosphatase with 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA for 4 hours at 37°C released < 0.1% of the total radioactivity.

Endonuclease Activity: Incubation of a 50 µl reaction containing 50 units of Antarctic Phosphatase with 1 µg of ϕX174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RFII as determined by agarose gel electrophoresis.

RNase Activity (Extended Digestion): A 10 µl reaction in Antarctic Phosphatase Reaction Buffer containing 40 ng of fluorescein labeled RNA transcript and 5 units of Antarctic Phosphatase is incubated at 37°C. After incubation for 16 hours, > 90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

Usage Notes:

Molecular Weight: Antarctic Phosphatase is a homodimer. The molecular weight of the monomer is 35 kDa.

Antarctic Phosphatase, as are most alkaline phosphatases, is a Zn^{2+} and Mg^{2+} –dependent enzyme and does requires supplemental zinc.

Adding 1/10 volume of 10X Antarctic Phosphatase Reaction Buffer will provide the amount of Zn^{2+} that the enzyme requires for activity.

Antarctic Phosphatase is also active in all restriction enzyme NEBuffers 1.1, 2.1, 3.1 and CutSmart® Buffer only when supplemented with 10X Antarctic Phosphatase Reaction Buffer.

Antarctic Phosphatase activity is enhanced in the presence of monovalent salts.

Antarctic Phosphatase is inhibited by metal chelators (e.g. EDTA), inorganic phosphate and phosphate analogs.

The Antarctic Phosphatase activity is decreased in the presence of reducing agents (DTT, β-ME).

References:

1. Rina, M. et al. (2000) *Eur. J. Biochem.* 267, 1230–1238.
2. Guthrie, E., unpublished observations

Companion Products Sold Separately:

Antarctic Phosphatase Reaction Buffer
#B0289S 6 ml

T4 DNA Ligase
#M0202S 20,000 units
#M0202L 100,000 units
#M0202T 20,000 units
#M0202M 100,000 units

Quick Ligation™ Kit
#M2200S 30 rxns
#M2200L 150 rxns

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Protocol for Dephosphorylation of 5′-ends of DNA using Antarctic Phosphatase in Restriction Enzyme Reaction

1. Digest 1–5 µg of plasmid DNA in a 20 µl reaction as follows:

DNA	≥ 1 µl
Restriction Enzyme Buffer (10X)	2 µl
Restriction Endonuclease	1 µl
H_2O, purified	to 20 µl

Note: Scale larger reaction volumes proportionally.

2. Incubate at 37°C for 60 minutes or follow manufacturer's recommendations.
3. Add 1/10 volume of 10X Antarctic Phosphatase Reaction Buffer and 5 units of Antarctic Phosphatase for every 1 pmol of DNA ends (about 1 µg of a 3 kb plasmid) and incubate at 37°C for 30 minutes.
4. Stop reaction by heat-inactivation of Antarctic Phosphatase and restriction enzyme (follow manufacturer's recommendations).

Page 2 (M0289)

Note: If restriction enzyme cannot be heat-inactivated, DNA purification is required before ligation.

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