Antarctic Phosphatase

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

M0289S

1,000 units	**5,000 U/ml**	**Lot: 0211312**
RECOMBINANT	**Store at –20°C**	**Exp: 12/15**

Description: Antarctic Phosphatase catalyzes the removal of 5´ phosphate groups from DNA and RNA. Since phosphatase-treated fragments lack the 5´ phosphoryl termini required by ligases, they cannot self-ligate (1). This property can be used to decrease the vector background in cloning strategies.

Heat Inactivated In 5 Minutes at 70°C

Source: An *E. coli* strain that carries the TAB5 AP gene, originally cloned in plasmid pNI (2), recloned in plasmid pEGTAB7–4.1(3).

Applications:

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

Supplied in: 10 mM Tris-HCl (pH 7.4), 1 mM $MgCl_2$, 0.01 mM $ZnCl_2$, 1 mM DTT 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 Pst I (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).

Vector Dephosphorylation Protocol:

1. Add 1/10 volume of 10X Antarctic Phosphatase Reaction Buffer to 1– 5 µg of DNA cut with any restriction endonuclease in any buffer.
2. Add 1 µl of Antarctic Phosphatase (5 units) and mix.
3. Incubate for 15 minutes at 37°C for 5´ extensions or blunt-ends, 60 minutes for 3´ extensions.
4. Heat inactivate for 5 minutes at 70°C (or as required to inactivate the restriction enzyme).
5. Proceed with ligation.

Usage Notes: Antarctic Phosphatase is also active in NEBuffers 1, 2, 3 or 4 as well as the NEBuffer for EcoRI **ONLY** when supplemented with 10X Antarctic Phosphatase Reaction Buffer to a final concentration of 1X. Adding 1/10 volume of 10X Antarctic Phosphatase Reaction Buffer will provide the amount of Zn^{2+} that the enzyme requires for activity.

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: Incubation of a 10 µl reaction containing 5 units of Antarctic Phosphatase with 40 ng of fluorescein labeled RNA transcript for 4 hours at 37°C resulted in no detectable degradation of the RNA as determined by gel electrophoresis using fluorescence detection.

CERTIFICATE OF ANALYSIS

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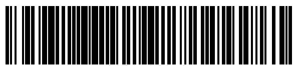

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RNase Activity: Incubation of a 10 µl reaction containing 5 units of Antarctic Phosphatase with 40 ng of fluorescein labeled RNA transcript for 4 hours at 37°C resulted in no detectable degradation of the RNA as determined by gel electrophoresis using fluorescence detection.

CERTIFICATE OF ANALYSIS

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

Transformation Assay: pUC19 was cleaved with HindIII, HincII or PstI, each purified by Qiaprep™ spin column and resuspended in water at 0.2 mg/ml. 1 µg of each DNA was treated with 5 units of Antarctic Phosphatase. Each reaction was carried out with 1 µg of DNA in a 50 µl reaction volume with 1X Antarctic Phosphatase Reaction Buffer for 30 minutes at 37°C, followed by heat inactivation at 65°C for 5 minutes.

Ligations were performed using the NEB Quick Ligation Kit protocol with 2.5 µl (50 ng) of vector DNA directly from the heat-inactivated phosphatase reaction mix. Inserts were included in 3-fold molar excess. Either HindIII cleaved fragments of λ DNA, HaeIII cleaved fragments of φX174 RF I DNA or PstI cleaved fragments of λ DNA were inserted as indicated.

5 ng of each ligation was transformed into *E. coli* DH5-α. The equivalent of 1.0 ng were plated on LB plates that contained IPTG, X-gal and ampicillin.

	Antarctic Phosphatase Units	Blue Colonies	White Colonies	Background Reduction (%)	Insert (%)
5´ Overhang					
HindIII cleaved and ligated	0	1360	0		
Phosphatase treated and ligated	5	16	0		
Phosphatase treated, insert ligated	5	24	216	98.2	15.9
Blunt End					
HincII cleaved and ligated	0	1028	0		
Phosphatase treated and ligated	5	0	0		
Phosphatase treated, insert ligated	5	24	92	97.7	8.9
3´ Overhang					
PstI cleaved and ligated	0	872	0		
Phosphatase treated and ligated	5	8	0		
Phosphatase treated, insert ligated	5	20	348	97.7	40.0

Heat Inactivation: 70°C for 5 minutes.

References:

1. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning*: *A Laboratory Manual*, (2nd ed.), (p. 5.72). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
2. Rina, M. et al. (2000) *Eur. J. Biochem.* 267, 1230–1238.
3. Guthrie, E., unpublished observations

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