# Antarctic Phosphatase

**Catalog:** RK20537
**Size:** 500 U / 1,000U / 5,000U
**Concentration:** 5,000 U/ml
**Components:**

| | |
|---|---|
| Antarctic Phosphatase (5,000 U/ml) | RM21308 |
| 10X Antarctic Phosphatase Reaction Buffer | RM20152 |

## 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:**
An *E. coli* strain that carries the TAB5 AP gene.

**Unit Definition:**
One unit is defined as the amount of enzyme that dephosphorylates 1 µg of pUC19 vector DNA cut with a restriction enzyme generating 5′ recessed ends in 30 minutes at 37°C. Dephosphorylation is defined as > 95% inhibition of recircularization in a self-ligation reaction and is measured by transformation into *E. coli*.

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

**Storage Temperature:** -20℃

**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 @ 25°C

**Heat Inactivation:** 80°C for 2 min

**Molecular Weights:** Apparent: 35 kDa. Theoretical: 69 kDa.

**Applications:**
Dephosphorylation, RNA Modification, *In vitro* Synthesis (IVT)

**Application Features**

- Dephosphorylation 5′ and 3′ ends of DNA and RNA.
- Dephosphorylation of cloning vector DNA to prevent recircularization during ligation.
- Dephosphorylation of DNA prior to end-labeling using T4 Polynucleotide Kinase.
- Treatment of dNTPs in PCR reactions prior to sequencing or SNP analysis.

## Instructions

➢ **Protocol for Dephosphorylation of 5′-ends of DNA using Antarctic Phosphatase**

1. Prepare a 20 µl reaction as follows:

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

2. Incubate at 37°C for 30 minutes.
3. Stop reaction by heat-inactivation at 80°C for 2 minutes.

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

*** Scale larger reaction volumes proportionally.*

**Dephosphorylation of 5´-ends of DNA in Restriction Enzyme Reaction**

- The phosphatase can be added directly into the digestion reaction during or after DNA digestion.
- 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 $Zn^{2+}$ required for enzyme activity.
- The restriction enzyme should be heat inactivated at the same time as the phosphatase after digest and dephosphorylation.
- If the restriction enzyme cannot be heat inactivated, DNA purification is required before ligation.

**Notes**

1. Adding 1/10 volume of 10X Antarctic Phosphatase Reaction Buffer provides the amount of $Zn^{2+}$ that the enzyme requires for activity.
2. Antarctic Phosphatase is also active in ABuffer A/B/C/S and CutA/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. Molecular Weight: Antarctic Phosphatase is a homodimer. The molecular weight of the monomer is 35 kDa.
6. Antarctic Phosphatase, as are most alkaline phosphatases, is a $Zn^{2+}$ and $Mg^{2+}$ –dependent enzyme and does require supplemental zinc.

**QC Process:**

- ◆ Purity is above 95% detected by SDS-PAGE.
- ◆ No endonucleases, ss-DNase, and other RNases contamination.
- ◆ No residual host genomic DNA is detected by PCR.