MediRes Corp.

USER GUIDE **Bi2M-AP**

Alkaline Phosphatase

Product #: Bi2M-AP
Quantity: 1,000 Units

Product Description

- **Source:**
  - Recombinant heat-labile alkaline phosphatase from *Escherichia coli* strain expressing a gene from Antarctic microorganisms (TAB5).
- **Function:**
  - Non-specifically catalyzes the removal of 5' or 3' terminal phosphate groups from DNA, RNA, dNTP, and rNTP.
- **Applications:**
  - Removes phosphorylated ends of DNA and RNA for cloning or probe end labeling.
  - Prevents self-ligation of linearized plasmid DNA in cloning.
  - Degrades unbound dNTPs in PCR for sequencing or SNP analysis.
- **Features:**
  - Rapid dephosphorylation.
  - Thermally unstable; inactivated by incubation at 80°C for 2 minutes.
- **Activity Definition:**
  - One unit is the amount of enzyme required to dephosphorylate 1 µg of linearized pUC19 vector after 30 minutes at 37°C, resulting in over 95% inhibition of vector self-ligation (measured by E. coli transformation).
- **Inactivation/Inhibition:**
  - Fully inactivated by heating at 80°C for 2 minutes.
  - Inhibited by metal ion chelating agents like EDTA.
- **Purity and Concentration:**
  - Purity ≥ 95% by SDS-PAGE.
  - Concentration: 5 u/µL.
- **Storage Buffer:**
  - 10 mM Tris-HCl, 1 mM $MgCl_2$, 0.01 mM $ZnCl_2$, 50% glycerol, pH 7.4.
- **10× ALP Reaction Buffer:**
  - 500 mM Bis-Tris-HCl, 10 mM $MgCl_2$, 1 mM $ZnCl_2$, pH 6.0.

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Storage

- Store at -20℃.

Components Provided

Alkaline Phosphatase (Thermosensitive)	200µL
10X ALP Reaction Buffer	1mL

Protocol

1. Prepare the reaction system as described.

COMPONENT	FINAL VOLUME/AMOUNT
Alkaline Phosphatase	1µL
10X ALP Reaction Buffer	2µL
Nuclease Free Water	To 20µL

2. Mix gently and centrifuge the liquid to the bottom of the tube.
3. Incubate at 37°C for 30 minutes.
4. For better dephosphorylation, extend incubation at 37°C for an additional 15-30 minutes (up to 60 minutes for optimal results).
5. Add 0.5 µL of 25 mM EDTA to stop the reaction.
6. Inactivate alkaline phosphatase by heating at 80°C for 2 minutes.
7. Use an appropriate DNA purification kit or DNA gel recovery kit for gel cutting recovery and purification after electrophoresis. Alternatively, use phenol-chloroform extraction, ethanol precipitation, or other methods to purify the digested and dephosphorylated DNA or RNA as needed for subsequent experiments.

NOTES:

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