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APex™ Heat-Labile Alkaline Phosphatase

Cat. Nos. AP49010, AP49050, and AP49100

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1. Introduction

APex™ Heat-Labile Alkaline Phosphatase is a novel alkaline phosphatase that dephosphorylates 5′ phosphates from a broad range of substrates including: DNA 5′ protruding, blunt, and 5′-recessed ends and RNA ends, using a single 10-minute protocol.[1] Isolated from a recombinant source, this proprietary enzyme is rigorously purified to be nuclease contamination-free for improved performance. APex Phosphatase is highly active. The enzyme maintains >90% of its activity after 90 minutes at 50°C, yet it is completely and irreversibly inactivated when heated at 70°C for 5 minutes. The half-life is <12 minutes at 55°C, <5 minutes at 60°C, and <3 minutes at 65°C. APex Phosphatase is also active in a broad range of reaction buffers, in which it does not require any buffer supplementation. For example, APex Phosphatase had >95% activity over a range of pH from 5.5-12, at ionic strength up to 1 M Na^+, NH_4^+, K^+, Cl^-, or $Acetate^-$, and in the presence of 10% Triton® X-100. Additionally it had full activity in all 21 restriction enzyme buffers tested.

The fast, complete, and irreversible heat-inactivation allows for easy transition to the next experimental step; no time-consuming substrate purification with phenol:chloroform extraction is required. Researchers can thus perform sequential steps including: restriction enzyme digestion, dephosphorylation, enzyme inactivation, and ligation or end-labeling in a single tube. This simplifies many experiments and minimizes the amount of nucleic acid required.

APex Heat-Labile Alkaline Phosphatase is available in 10-, 50-, and 100- reaction sizes. A 10X Reaction Buffer is also provided.

Applications

- Dephosphorylation of DNA vectors prior to cloning to prevent recircularization.
- Preparation of 5′-nucleic acid termini for 5′-end labeling with polynucleotide kinase.
- Dephosphorylation of DNA or RNA.

2. Product Specifications

Storage: Store only at –20°C in a freezer without a defrost cycle.

Storage Buffer: APex Phosphatase is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM zinc acetate, 10 mM $MgCl_2$, and Triton X-100.

Activity: 1 microliter of APex Heat-Labile Phosphatase dephosphorylates 1 µg of pUC19 vector DNA digested with *Hin*d III (5′ protruding), *Hin*c II (blunt), or *Pst* I (5′ recessed) in 10 minutes at 37°C.

10X Reaction Buffer: 330 mM Tris-acetate (pH 7.5), 660 mM potassium acetate, 100 mM magnesium acetate, and 5 mM dithiothreitol.

Quality Control: APex Phosphatase is function-tested to meet our Cloning Quality Standard of >99% inhibition of *Pst* I-cut vector self-ligation as assayed by *E. coli* transformation, when treating 1 µg of pUC19 DNA with 1 µl of enzyme for 10 minutes 37°C.

Contaminating Activity Assays: APex Phosphatase is free of detectable DNA exo- and endonuclease, and RNase activities.

3. Related Products

The following products are also available:

- Fast-Link™ DNA Ligation Kits
- T4 Polynucleotide Kinase
- Tobacco Acid Pyrophosphatase
- TransforMax™ EC100™ Electrocompetent and Chemically Competent *E. coli*
- TransforMax™ EC100™-T1^{R} Phage T1-Resistant Electrocompetent and Chemically Competent *E. coli*

4. Suggested Protocol for Vector Dephosphorylation

The following protocol describes the linearization (by restriction enzyme digestion) and APex Phosphatase-mediated dephosphorylation of the vector DNA. APex Phosphatase reactions can also be performed directly in most restriction enzyme (RE) buffers without the need for buffer supplemention. The enzyme is effective on blunt, 5′- and 3′-overhang ends regardless of the length of the overhang. Use the protocol below for any type of DNA end and RE buffer.

Note: *APex Phosphatase is not active on capped mRNA. Capped mRNA first requires treatment with Tobacco Acid Pyrophosphatase in order to remove the cap, thus generating a decapped 5′-phosphorylated terminus which can be treated with APex Phosphatase.*

Protocol

1. Digest vector DNA with restriction enzyme(s) and RE buffer(s) of choice, as per manufacturer's recommendations.
2. To the completed restriction digest, directly add 1 µl of APex Heat-Labile Phosphatase.
3. Incubate at 37°C for 10 minutes.
4. Heat at 70°C for 5 minutes to inactivate the APex Phosphatase.
5. Mix appropriate amounts of dephosphorylated vector DNA and the DNA to be cloned.
6. Ligate and transform bacterial cells using standard protocols.

   Epicentre's Fast-Link DNA Ligation Kit (available separately) performs cohesive-end ligations in 5 minutes and blunt-end ligations in 15 minutes.

   Epicentre's TransforMax EC100 Electrocompetent *E. coli* (available separately) have a transformation efficiency of >1 x 10^{10} cfu/µg and are ideal for this application.

Optional: Vector DNA restriction and dephosphorylation can be performed simultaneously with restriction enzymes having 37°C optimal temperatures. Just add 1 µl of APex Heat-Labile Phosphatase directly to your standard restriction enzyme reaction and incubate as per restriction enzyme requirements. Proceed to step 4 afterwards.

5. References

1. Meis, R. (2004) *Epicentre Forum* **11** (5) 4.

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