# Thermosensitive Alkaline Phosphatase

# Instruction for Use

**Cat. No./Spec.: E1013-A/300 U; E1013-B/1,000 U; E1013-C/5,000 U**

**Concentration: 1 U/μL**

## Product Description

*Thermosensitive Alkaline Phosphatase is a novel alkaline phosphatase that exhibits activity in commonly used restriction endonuclease buffers as well as PCR buffers. This enzyme catalyzes the release of 5' and 3' phosphate groups in DNA, RNA, and nucleotides. Additionally, it can remove phosphate groups from proteins. Thermosensitive Alkaline Phosphatase is capable of dephosphorylating all types of DNA termini within 10 minutes at 37° C. The enzyme is inactivated by heat at 75° C within 5 minutes. Consequently, there is no need to remove the alkaline phosphatase before ligation.*

## Components

| *Component* | *E1013-A* | *E1013-B* | *E1013-C* |
|---|---|---|---|
| *Thermosensitive Alkaline Phosphatase (1 U/μL)* | *300 μL* | *1 ml* | *1 ml × 5* |
| *10X DSAP Buffer* | *1.25 ml* | *1.25 ml × 2* | *1.25 ml × 10* |

## Storage Condition & Shelf Life

*Store at -20°C. The product is valid for 2 years.*

## Source

*Recombinant E. coli strain containing the alkaline phosphatase gene cloned from bacteria.*

## Unit Definition

*One unit is defined as the amount of enzyme required to dephosphorylate the 5'-ends of 1 μg of linearized pUC57 DNA in DSAP Buffer at 37°C within 10 minutes.*

## Scope of Application

*- Dephosphorylation of cloned vector DNA to prevent re-circularization during ligation.*

*- Simultaneous enzymatic cleavage and dephosphorylation of vector DNA.*

*- Purification of PCR products: nucleotide degradation of PCR products before sequencing.*

*- Depehosphorylation of the 5'-ends of nucleic acids before T4 polynucleotide kinase labeling.*

*- Other applications requiring dephosphorylation of DNA and RNA substrates.*

*- Dephosphorylation of proteins.*

## Protocol

*1. Prepare reaction system.*

| *Component* | *Amount* |
|---|---|
| *Linear DNA (~3 kb plasmid)* | *1 μg (~1 pmol termini)* |
| *Thermosensitive Alkaline Phosphatase (1 U/μL)* | *1 μL* |
| *10X DSAP Buffer* | *2 μL* |
| *dd$H_2O$* | *To 20 μL* |

*2. Mix thoroughly and incubate at 37° C for 10 minutes.*

*3. Heat at 75° C for 5 minutes to terminate the reaction.*

## Notes

*1. The binding of Thermosensitive Alkaline Phosphatase to DNA may cause band shifting in agarose gels. To avoid this, you can use 6X DNA Loading Dye & SDS solution to incubate the samples at 65°C for 10 minutes, and then cool on ice before electrophoresis.*

*2. Thermosensitive Alkaline Phosphatase is active in all restriction enzyme buffers and can be directly added to the digested DNA. There is no need to heat inactivate the restriction enzymes before dephosphorylation treatment.*

*This product is for research use only.*