FastAP™ Thermosensitive Alkaline Phosphatase

Catalog Number EF0651, EF0652, EF0654

Pub. No. MAN0012876 **Rev.** C.00

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Sheets (SDSs) are available from **thermofisher.com/support.**

Contents and storage

Cat. No.	Contents	Amount	Storage
EF0651	FastAP™ Thermosensitive Alkaline Phosphatase	1000 U for 1000 reactions, 1 U/µL	-25 °C to -15 °C
	10X FastAP™ Buffer	2 x 1.5 mL	
EF0652	FastAP™ Thermosensitive Alkaline Phosphatase	5 x 1000 U for 5000 reactions, 1 U/µL	
	10X FastAP™ Buffer	10 x 1.5 mL	
EF0654	FastAP™ Thermosensitive Alkaline Phosphatase	300 U for 300 reactions, 1 U/µL	
	10X FastAP™ Buffer	1.5 mL	

BSA included

Description

Thermo Scientific™ FastAP™ Thermosensitive Alkaline Phosphatase catalyzes the release of 5'- and 3'- phosphate groups from DNA, RNA and nucleotides. This enzyme also removes phosphate groups from proteins. FastAP™ is a novel alkaline phosphatase, which is active in all Thermo Scientific restriction enzyme buffers as well as in PCR buffers. It dephosphorylates all types of DNA ends in 10 min at 37 °C. The enzyme is inactivated in 5 min at 75 °C. Therefore, removal of alkaline phosphatase is not required prior to ligation.

Applications

- Dephosphorylation of cloning vector DNA to prevent recircularization during ligation.
- Simultaneous digestion and dephosphorylation of vector DNA.
- PCR product clean-up: nucleotide degradation prior to sequencing of PCR product.
- Dephosphorylation of nucleic acid 5'-termini prior to labeling with T4 Polynucleotide Kinase.
- Other applications where dephosphorylation of DNA and RNA substrates is necessary.
- Protein dephosphorylation.

Source

E.coli cells with a cloned bacterial AP gene.

Definition of Activity Unit

One unit is the amount of the enzyme required to dephosphorylate 5'-termini of 1 µg of linearized pUC57 DNA in 10 min at 37 °C in FastAP™ buffer.

Storage Buffer

The enzyme is supplied in: 20 mM HEPES-NaOH (pH 7.4), 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, 0.1 % (v/v) Triton X-100 and 50 % (v/v) glycerol.

10X FastAP Buffer

100 mM Tris-HCl (pH 8.0 at 37 °C), 50 mM $MgCl_2$, 1 M KCl, 0.2 % Triton X-100 and 1 mg/mL BSA.

Inhibition and Inactivation

- Inhibitors: metal chelators.
- Inactivated by heating at 75 °C for 5 min.

Note

- Binding of FastAP™ Thermosensitive Alkaline Phosphatase to DNA may result in a band shift in agarose gels. To avoid this, incubate samples with 6X Loading Dye & SDS Solution (#R1151) at 65 °C for 10 min and chill on ice prior to electrophoresis.
- FastAP™ Thermosensitive Alkaline Phosphatase is active in all restriction enzyme buffers and may be added directly to digest DNA. Heat inactivation of the restriction enzyme before dephosphorylation reaction is not necessary.

Protocol for fast simultaneous plasmid vector linearization and dephosphorylation

1. Prepare the following reaction mixture containing:

Components	Volume
Plasmid DNA	1 µg
10X Thermo Scientific™ FastDigest™ Buffer	2 µL
FastDigest™ Restriction Enzyme	1 µL
FastAP™ Thermosensitive Alkaline Phosphatase	1 µL
Water, nuclease-free (#R0581)	to 20 µL
Total volume	20 µL

2. Mix thoroughly, spin briefly and incubate at 37 °C for 10 min.
3. Stop reactions by heating at 65 °C for 15 min or at 80 °C for 20 min (if restriction enzyme is not inactivated at 65 °C).

Note. For FastDigest™ SphI (PaeI) (#FD0604), simultaneous digestion and dephosphorylation is not recommended. Perform digestion, spin column purification and then dephosphorylation.

Protocol for nucleic acid dephosphorylation

This protocol is suitable for removal of 3' and 5' -phosphate groups from DNA and RNA.

1. Prepare the following reaction mixture:

Components	Volume
Linear DNA (~3 kb plasmid)	1 µg (~1 pmol termini)
10X reaction buffer for AP used in reaction	2 µL
FastAP™ Thermosensitive Alkaline Phosphatase	1 µL (1 U)
Water, nuclease-free (#R0581)	to 20 µL
Total volume	20 µL

2. Mix thoroughly, spin briefly and incubate 10 min at 37 °C.
3. Stop reaction by heating for 5 min at 75 °C.

Note. For efficient dephosphorylation plasmid DNA should be free of RNA and genomic DNA.

Protocol for dephosphorylation of proteins

Reaction mixture: 1X FastAP™ reaction buffer, 0.1-0.2 mg/mL of phosphoprotein, 10 U of FastAP™ Thermosensitive Alkaline Phosphatase. Incubate at 37 °C for 1 h.

For example: If you are doing a 20 µL reaction setup you need 2 µL 10X FastAP™ buffer, 2-4 µg of protein (to be in the range of 0.1-0.2 mg/mL) and 10 U of FastAP™ Thermosensitive Alkaline Phosphatase (1 U/µL).

Note

- The reaction can be stopped by addition of a final concentration of 50 mM EDTA (#R1021) or by addition of a final concentration of 10 mM sodium orthovanadate (Na_3VO_4).
- The optimal incubation time and the enzyme concentration must be determined experimentally for each substrate.

Limited product warranty

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