Fermentas
LIFE SCIENCES

CERTIFICATE OF ANALYSIS

FastAP™ Thermosensitive Alkaline Phosphatase

#EF0651 1000 u for 1000 reactions

Lot: **Expiry Date:**

Concentration: 1 u/µl
Supplied with: 2 x 1.5 ml of 10X FastAP™ Buffer

Store at -20°C

In total 3 vials. BSA included

ISO 9001 ISO 14001
www.fermentas.com

Description

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 Fermentas 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 chil on ice prior to electrophoresis.
- FastAP™ Thermosensitive Alkaline Phosphatase is active in all restriction enzyme buffers and may be added directly to digested DNA. Heat inactivation of the restriction enzyme before dephosphorylation reaction is not necessary.

QUALITY CONTROL ASSAY DATA

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 10 u of FastAP™ with 1 µg of pUC19 DNA in 50 µl of FastAP™ buffer for 4 hours at 37°C.

Ribonuclease Assay

≥0.5% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 10 u of FastAP™ with 1 µg of [^{3}H]-RNA in 50 µl of FastAP™ buffer for 4 hours at 37°C.

Labeled Oligonucleotide (LO) Assay

Single-stranded and double-stranded oligonucleotides were incubated with 10 units of FastAP™ for 4 hours at 37°C and then 5'-[^{32}P]-labeled with T4 Polynucleotide Kinase.
No detectable degradation of oligonucleotides was observed after separation on a polyacrylamide gel and phosphorimaging analysis.

Quality authorized by:  Jurgita Zilinskiene

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Protocol for fast simultaneous plasmid vector linearization and dephosphorylation

1. Prepare the following reaction mixture containing:

Plasmid DNA	1 µg
10X FastDigest® Buffer	2 µl
FastDigest® Restriction Enzyme	18 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) (#FD0601), simultaneous digestion and dephosphorylation is not recommended. Perform digestion, spin column purification and then dephosphorylation.

Protocol for dephosphorylation of DNA 5'-termini

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

1. Prepare the following reaction mixture:

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.

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.