M5 Thermosensitive Alkaline Phosphatase User Manual

Products	Units	Cat#.
M5 Thermosensitive Alkaline Phosphatase	200U	MF401-01
M5 Thermosensitive Alkaline Phosphatase	1000U	MF401-05

【Storage】
-20˚C

【Description】
M5 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.

【Application】
1) Dephosphorylation of cloning vector DNA to prevent recircularization during ligation.
2) Simultaneous digestion and dephosphorylation of vector DNA.
3) PCR product clean-up: nucleotide degradation prior to sequencing of PCR product.
4) Dephosphorylation of nucleic acid 5’-termini prior to labeling with T4 Polynucleotide Kinase.
5) Other applications where dephosphorylation of DNA and RNA substrates is necessary.
6) Protein dephosphorylation.

【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.

【Components】

	MF401-01	MF401-05
M5 Thermosensitive Alkaline Phosphatase (1U/µl)	200µl	1000µl
10X M5 AP Buffer*	500µl	2x1.5ml

*10X M5 AP 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.
M5 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.

【Protocol】

A. Protocol for fast simultaneous plasmid vector linearization and dephosphorylation

1. Prepare the following reaction mixture containing:

Plasmid DNA	1 µg
10X Restriction Enzyme Buffer	2 µL
Restriction Enzyme	1 µL
M5 Thermosensitive Alkaline Phosphatase	1 µL
Water	to 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).

B. 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:

Linear DNA (~3 kb plasmid)	1 µg (~1 pmol termini)
10X M5 AP Buffer	2 µL
M5 Thermosensitive Alkaline Phosphatase	1 µL (1 U)
Water, nuclease-free	to 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.

C. Protocol for dephosphorylation of proteins

Reaction mixture: 1X M5 AP reaction buffer, 0.1-0.2 mg/mL of phosphoprotein,
10 U of M5 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 M5 AP buffer, 2-4 µg of protein (to be in the range of 0.1-0.2 mg/mL) and 10 U of M5 Thermosensitive Alkaline Phosphatase (1 U/µL).