USB® Shrimp Alkaline Phosphatase

Product number 78390

Dephosphorylation protocol

Shrimp Alkaline Phosphatase (SAP) removes phosphate groups from the 5'-ends of DNA and RNA which is useful in preventing re-ligation of linearized plasmid for cloning applications, as well as end-labeling of probes. SAP also dephosphorylates dNTPs which allows for efficient removal of unincorporated nucleotides from PCR products prior to DNA sequencing or SNP analysis.

SAP has approximately the same specific activity as Calf Intestinal Alkaline Phosphatase (CIAP), and like CIAP, is active in virtually all restriction enzyme reaction buffers. Unlike CIAP, SAP is completely and irreversibly inactivated by heating reactions at 65°C for 15 minutes.

10X Shrimp Alkaline Phosphatase Reaction Buffer (included with the enzyme): 200 mM Tris-HCl, pH 8.0 and 100 mM $MgCl_2$

SAP and 10X Shrimp Alkaline Phosphatase Reaction Buffer have been functionally tested in the following protocol:

Protocol for dephosphorylation of 5'-ends of DNA:

1. Resuspend 1 pmol of DNA ends (about 1 µg of a 3 kb plasmid) in nuclease-free water.
2. Prepare reaction mix in a 20 µl volume according to the following table:

DNA	> 1 µl
10X SAP Reaction Buffer	2 µl
Water, Nuclease-Free	up to 19 µl
SAP (1 unit/µl)	1 µl

Note: Scale larger reaction volumes proportionally.

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3. Incubate at 37°C for 30-60 minutes.
4. Stop reaction by heating at 65°C for 15 minutes. This completely inactivates SAP.

Notes: The minimum effective amount of SAP for dephosphorylation of 1 pmol of DNA termini in 1 hour at 37°C is:

0.05 units for 5-protruding termini
0.05 units for blunt termini
0.1 units for 5'-recessed termini

Protocol for dephosphorylation of 5'-ends of DNA in restriction enzyme reaction:

1. Digest 1-5 µg of plasmid DNA in a 20 µl volume according to the following table:

DNA	> 1 µl
10X Restriction Enzyme Buffer	2 µl
Water, Nuclease-Free	up to 19 µl
Restriction Endonuclease	1 µl

Note: Scale larger reaction volumes proportionally.

2. Incubate at 37°C for 60 minutes.
3. Add 1 unit of SAP for every 1 pmol of DNA ends (about 1 µg of a 3 kb plasmid) and incubate at 37°C for 30-60 minutes.
4. Stop reaction by heating at 65°C for 15 minutes. This completely inactivates SAP.

Note: Some restriction enzymes require 80°C for complete heat-inactivation. Follow manufacturers' recommendations.

Affymetrix, Inc.
USA: USBtechsupport@affymetrix.com
Europe: USBtechsupporteurope@affymetrix.com

usb.affymetrix.com
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