Product Contents

Part# 9PIM820

Revised 3/07

Shrimp Alkaline Phosphatase (SAP), Blue/White Cloning Qualified:

Part No.	Conc. (u/µl)	Size (units)
M820A	1	500

Description: Shrimp Alkaline Phosphatase (SAP) catalyzes the dephosphorylation of 5´ phosphates from DNA. Unlike Calf Intestinal Alkaline Phosphatase, SAP is completely and irreversibly inactivated by heating at 65°C for 15 minutes. Alkaline phosphatases are used to prevent recircularization and religation of linearized cloning vehicle DNA by removing phosphate groups from both 5´-termini and may also be used for the dephosphorylation of 5´ phosphorylated ends of DNA for subsequent labeling with [^{32}P]ATP and T4 Polynucleotide Kinase. SAP is active on 5´ overhangs, 5´ recessed and blunt ends (1).

Enzyme Storage Buffer: Shrimp Alkaline Phosphatase (SAP) is supplied in 25mM Tris-HCl (pH 7.6 at 4°C), 1mM $MgCl_2$, 0.1mM $ZnCl_2$ and 50% glycerol.

Shrimp Alkaline Phosphatase 10X Reaction Buffer (M821A): When the 10X Reaction Buffer supplied with this enzyme is diluted 1:10, it has a composition of 50mM Tris-HCl (pH 9.0 at 37°C) and 10mM $MgCl_2$.

Source: Shrimp (*Pandalus borealis*).

Unit Definition: One unit is defined as the amount of enzyme required to catalyze the hydrolysis of 1µmol of 4-nitrophenyl phosphate per minute at 37°C in 1M diethanolamine, 10.9mM 4-nitrophenyl phosphate, 0.5mM $MgCl_2$ (pH 9.8). See the unit concentration on the Product Information Label.

Storage Temperature: See the Product Information Label for storage recommendations. Avoid multiple freeze-thaw cycles. See the expiration date on the Product Information Label.

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Quality Control Assays

Contaminant Activity

Endonuclease Assay: To test for endonuclease activity, 1µg of Type I supercoiled plasmid DNA is incubated with 5 units of Shrimp Alkaline Phosphatase in 1X Reaction Buffer for one hour at 37°C. Following incubation, the supercoiled DNA is visualized on an ethidium bromide-stained agarose gel to verify the absence of visible nicking or cutting.

DNase and RNase Assay: To test for nuclease activity, 50ng of radiolabeled DNA or radiolabeled RNA is incubated with 5 units of Shrimp Alkaline Phosphatase in 1X Reaction Buffer for one hour at 37°C, and the release of radiolabeled nucleotides is monitored by scintillation counting of TCA-soluble material. Minimum passing specification is ≤3% release for DNase and ≤3% release for RNase.

Blue/White Assay: pGEM®-3Zf(+) Vector is digested with representative restriction enzymes (leaving 5´-termini, 3´-termini or blunt ends). Each microgram of cut plasmid is treated with 2 units of Shrimp Alkaline Phosphatase for 1 hour at 37°C, kinased and ligated. The religated plasmid is then transformed into JM109 cells that are plated on X-Gal/IPTG/Ampicillin plates. White colonies result from transformation with ligated plasmids with damaged ends. These white colonies represent the number of false positives expected in a typical cloning experiment. Enzymes that generate overhangs must produce fewer than 2% white colonies, and blunt-cutting enzymes must produce fewer than 5% white colonies (2).

References

1. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
2. Hung, L. *et al.* (1991) A blue/white cloning assay for quality control of DNA restriction and modifying enzymes. *Promega Notes* **33**, 12–13.

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Usage Information

I. Reaction Conditions

A. Standard Dephosphorylation and Ligation Reaction

1. Incubate SAP (1 unit/µg DNA) with restriction-digested vector (restriction enzyme and buffer removed, DNA in water or TE buffer) at 37°C for 15 minutes in 1X SAP reaction buffer in a final volume of 30–50µl. This is a sufficient amount of SAP to completely dephosphorylate the vector regardless of overhang type (5´, 3´ or blunt).
2. Inactivate SAP by heating to 65°C for 15 minutes.
3. Centrifuge the reaction mix. Remove a 1–2µl aliquot for ligation with appropriate DNA insert in 1X ligation buffer at 4°C overnight for all types of DNA overhangs. Final volume should be between 10–50µl. Molar ratios for vector to insert can range between 1:8 to 8:1. A 1:1 ratio is recommended as a starting point.
4. Transform the ligated material directly into competent *E. coli* cells.

B. Streamlined Restriction Digestion, Dephosphorylation and Ligation Procedure

1. Combine restriction digestion and dephosphorylation of DNA vector in 1X restriction enzyme buffer. Use 15 units of restriction enzyme/µg vector and 10 units SAP/µg vector in a final volume of 30–50µl. Incubate at 37°C for 15 minutes. This is a sufficient amount of SAP to completely dephosphorylate the vector regardless of overhang type (5´, 3´ or blunt).
2. Heat-inactivate both restriction enzyme and SAP for 15 minutes at 65°C.
   Note: Not all restriction enzymes can be heat inactivated.
3. Centrifuge and remove 1–2µl (~40ng) of vector for ligation with appropriate DNA insert using 2X Rapid Ligation Buffer (Cat.# C6711) and 2µl (6 units) of T4 DNA Ligase (Cat.# M1801) at 15°C for 5 minutes (3´ or 5´ ends) or 15 minutes for blunt ends in a final reaction volume of 10–50µl. We recommend starting with a 1:2 molar ratio of vector:insert DNA.
4. Transform the ligated material directly into competent *E. coli* cells.

C. Standard Dephosphorylation and Labeling Reaction Using T4 Polynucleotide Kinase

1. Incubate SAP (1 unit/0.5pmol ends) and DNA at 37°C for 15 minutes in 1X SAP buffer in a final volume of 30–100µl.
2. Purify the DNA using the Wizard® DNA Clean-Up System (Cat.# A7280).
3. Labeling is carried out by incubating 8–10 units T4 Polynucleotide Kinase (M4101), ≤10pmol DNA ends and 15µl [γ^{32}P] ATP (3,000Ci/mmol, 10mCi/ml) at 37°C for 10 minutes in 1X PNK buffer and a final volume of 50µl.

II. Composition of Buffers and Solutions

2X Rapid Ligation Buffer

60mM	Tris-HCl (pH 7.8 at 25°C)
20mM	$MgCl_2$
2mM	ATP
10%	PEG
20mM	DTT

III. Related Products

Product	Size	Cat.#
Wizard® DNA Clean-Up System	100 preps	A7280
T4 Polynucleotide Kinase	100u	M4101
2X Rapid Ligation Buffer	3 × 500µl	C6711
T4 DNA Ligase	1,000u	M1801

Table 1. Activity of Shrimp Alkaline Phosphatase (Part# M820A) in Restriction Enzyme Buffers vs. 1X Shrimp Alkaline Phosphatase Buffer.

Promega Restriction Enzyme Buffer	% Activity of SAP
A	20
B	20
C	25
D	35
E	20
F	60
G	30
H	30
J	25
K	20
L	30
MULTI-CORE™	10

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