Shrimp Alkaline Phosphatase (rSAP), 2 U/µl

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR1101601	1000 U	500 µl Shrimp Alkaline Phosphatase

COMPONENT	COMPOSITION
Shrimp Alkaline Phosphatase	Shrimp Alkaline Phosphatase (rSAP), 2 U/µl, in storage buffer containing 50% (v/v) glycerol.
STORAGE	-20°C (until expiry date – see product label)

FEATURES

- Quickly and easily removes 5' phosphates from DNA, RNA, dNTPs and proteins
- Completely inactivated after 5 min at 65°C
- Active in common restriction enzyme and PCR buffers
- Exhibits excellent stability at 4°C and room temperature

APPLICATIONS

- Removal of nucleotides from PCR prior to sequencing
- Dephosphorylation of restriction-digested vectors to prevent religation prior to cloning
- Rapid dephosphorylation of RNA, proteins or other biomolecules

DESCRIPTION

biotechrabbit™ Shrimp Alkaline Phosphatase is a multipurpose alkaline phosphatase that dephosphorylates most biomolecules and is fully inactivated by heating for 5 min at 65°C. The enzyme is stable at room temperature and active in common buffers for restriction digestion and PCR.

Dephosphorylation of restriction-digested plasmid to prevent religation prior to cloning

Shrimp Alkaline Phosphatase provides an efficient method to reduce the religation of vectors during cloning of DNA fragments by dephosphorylating restriction digested plasmids. The enzyme dephosphorylates all types of DNA termini – 3'-protruding, blunt and 5'-protruding – and is either added to the restriction mixture or after digestion. Subsequently, Shrimp Alkaline Phosphatase is efficiently heat-inactivated.
During the ligation of plasmid with DNA fragments, the background of unwanted “empty” clones is reduced to less than 5%.

PCR cleanup prior to sequencing

The combination of Exonuclease 1 (not included) and Shrimp Alkaline Phosphatase provides a simple and fast method for PCR cleanup. Both enzymes are simply added to the PCR mixture, incubated and heat-inactivated. Shrimp Alkaline Phosphatase dephosphorylates nucleotides and primers, while Exonuclease 1 degrades primers, leaving a PCR product that can be directly used for sequencing or genotyping.

PROTOCOL

Prevention of contamination

When assembling the reactions, care should be taken to eliminate the possibility of contamination with undesired DNA and nucleases.

- Use separate clean areas for preparation of samples and reaction mixtures.
- Wear fresh gloves. Use sterile tubes and pipette tips with aerosol filters for assay setup.
- Use only water and reagents that are free of DNA and nucleases.
- With every assay setup, perform a contamination control reaction that does not include a template.

Assay requirements for Shrimp Alkaline Phosphatase

- Optimum working range for Shrimp Alkaline Phosphatase is between pH 7-9.
- The enzyme is active in most restriction and PCR buffers.
- Mg^{2+} (>1 mM) is required for activity.

Short protocol steps

- Add Shrimp Alkaline Phosphatase to PCR amplification or restriction reaction, or other biomolecule mixtures.
- Incubate at 37°C for 15 min (i.e. the last 15 min of the restriction reaction).
- Heat inactivate at 65°C for 5 min.

PROTOCOL FOR DEPHOSPHORYLATING RESTRICTION-DIGESTED PLASMID PRIOR TO CLONING

PROCEDURE	NOTES
Dephosphorylation during restriction digestion	
• Transfer 1 µg plasmid to a reaction tube. • Add 5 units restriction enzyme. • Add restriction enzyme buffer to a final 1× concentration. • Add 0.5 µl Shrimp Alkaline Phosphatase (1 U). • Add distilled water to a final volume of 50 µl. • Incubate at 37°C for 1 hour.	• Volumes may be scaled proportionally.
• Heat inactivate as recommended for the restriction enzyme.	• Shrimp Alkaline Phosphatase is inactive by the heat treatment.
• Proceed with ligation.	• For short-term storage, keep DNA at 4°C, for long-term storage at–20°C.
Fast dephosphorylation after restriction digestion of cloning vector	
• Digest plasmid according to the manufacturer's protocol. • Add 2.5 µl Shrimp Alkaline Phosphatase (5 U) for each microgram plasmid to the restriction mix. • Incubate at 37°C for 5 min. • Heat inactivate as recommended for the restriction enzyme.	• Shrimp Alkaline Phosphatase is inactive by the heat treatment.
• Proceed with ligation.	• For short-term storage, keep DNA at 4°C, for long-term storage at–20°C.

PROTOCOL FOR PCR CLEANUP PRIOR TO SEQUENCING

PROCEDURE	NOTES
• Transfer 5 µl of a completed amplification reaction to a new reaction tube. • Add 1 µl Shrimp Alkaline Phosphatase (2 U). • Add 10 units Exonuclease 1 (not included). • Incubate at 37°C for 15 min.	• Volumes may be scaled proportionally. • Heating steps may conveniently be performed in a suitably programmed thermocycler.
• Heat inactivate at 80°C for 15 min.	
• The reaction is now ready for sequencing or genotyping.	• For short-term storage, keep reaction mix at 4°C, for long-term storage at–20°C.

CERTIFICATE OF ANALYSIS

Unit Definition

One unit of Shrimp Alkaline Phosphatase release 1 µmol phosphate/min from 4-nitrophenyl phosphate in 0.1 M glycine-NaOH pH 10.4, 1 mM $MgCl_2$, 1 mM $ZnCl_2$ and 6 mM 4 nitrophenyl phosphate.

Quality Control

Protein Purity

Purified to apparent homogeneity by SDS-PAGE. No nuclease activity is detected.

Quality confirmed by: Head of Quality Control

SAFETY INSTRUCTIONS

For safety instructions please see Safety Data Sheets (SDS)/Sicherheitshinweise finden Sie in den SDS unter: http://www.biotechrabbit.com/support/documentation.html.

USEFUL HINTS

- Visit Applications at www.biotechrabbit.com for more products and product selection guides.
- Most biotechrabbit products are available in custom formulations and bulk amounts.

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