product codes:
E70092Y, E70092Z, E70092X

Shrimp Alkaline Phosphatase

Removes 5'-phosphates from DNA and RNA

Shrimp Alkaline Phosphatase (SAP) is a Tested User Friendly™ enzyme from USB Corporation. It is a high specific activity, heat-labile alkaline phosphatase that is useful in many applications, including preparing PCR products for labelling, cloning, sequencing (1–3), and SNP analysis. SAP is easily inactivated by heat and offers one-step degradation of dNTPs.

Dephosphorylation

Alkaline phosphatases can be used for the dephosphorylation of 5'-phosphorylated ends of DNA or RNA for subsequent labelling with ^{32}P using [γ-^{32}P]ATP, T4 polynucleotide kinase, and OptiKinase™. Dephosphorylation also prevents religation of linearized plasmid DNA in cloning experiments. Shrimp Alkaline Phosphatase has approximately the same specific activity as the calf intestine enzyme (800–1000 units/mg at 25 °C, pH 9.6) but, unlike the calf enzyme, SAP can be completely and irreversibly inactivated by heating for 15 min at 65 °C (Fig. 1). No further treatment is necessary.

Shrimp Alkaline Phosphatase is particularly useful in preparing PCR products for downstream applications involving sequencing, SNP analysis, or labelling methods. Typically, the excess dNTPs remaining after PCR interfere with subsequent reactions involving DNA synthesis. Shrimp Alkaline Phosphatase eliminates this problem by dephosphorylating all remaining dNTPs from the PCR mixture in one easy step.

For PCR cleanup, Shrimp Alkaline Phosphatase may be combined with Exonuclease I for removal of residual primers and extraneous single-stranded DNA reaction products. Hence, the use of alternative purification methods, such as columns, gels or magnetic separations, are completely eliminated. For convenience, refer to ExoSAP-IT™, which includes both enzymes in a format that is ready to use.

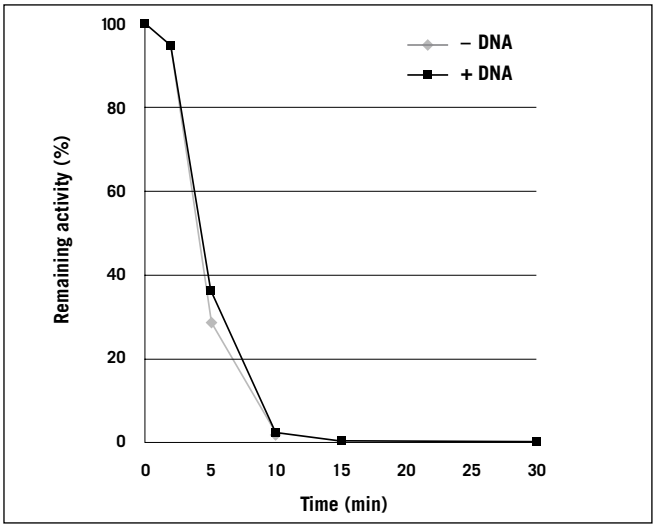

Fig 1. SAP inactivation at 65 °C. Reactions were set up using 20 units SAP (excess) with or without 2 µg lambda DNA *Eco*R I/*Hin*d III fragments. The reaction was incubated at 65 °C to inactivate the SAP. Aliquots from the reaction were placed on ice at selected time intervals and assayed for activity in the standard assay.

Specifications

Shrimp Alkaline Phosphatase is supplied with 10× reaction buffer and SAP dilution buffer; 1 ml each.

Tested User Friendly Functional Test: Dephosphorylation of a restriction enzyme digested plasmid (5–20 pmol of 5' ends, 0.1–0.5 units/pmol 5' ends). Reduces re-ligation to < 0.5% compared to the untreated control.

Purity: This enzyme is purified to apparent homogeneity and is free of all contaminating endonucleases, exonucleases, and ribonucleases.

Storage buffer: 25 mM Tris-HCl (pH 7.5), 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, 50% glycerol.

Concentration: 1 unit/ul

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Reference

1. Ruan, C. C., *et al*. *Comments* **17**, No. 1, United States Biochemical Corporation (1990).

2. Werle, E. *et al*. Convenient single-step, one tube purification of PCR products for direct sequencing. *Nucleic Acids Res* **22**, 4354–4355 (1994).

3. Hanke M. and Wink M. Direct DNA sequencing of PCR-amplified vector inserts following enzymatic degradation of primer and dNTPs. *Biotechniques* **17**, 858–860 (1994).

Ordering information

Shop online at www.amershambiosciences.com

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Shrimp Alkaline Phosphatase	5000 units	E70092X
Exonuclease I	2500 units	E70073Z
Exonuclease I	5000 units	E70073X
ExoSAP-IT	100 reactions	US78200
ExoSAP-IT	500 reactions	US78201
ExoSAP-IT	2000 reactions	US78202

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