illustra™ Shrimp Alkaline Phosphatase

illustra Shrimp Alkaline Phosphatase (SAP) removes 5′-phosphates from DNA and RNA. It is a high specific activity, heat-labile alkaline phosphatase that is useful in many applications, including preparing PCR products for labeling, cloning, sequencing, 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 labeling with ^{32}P using [γ-^{32}P]ATP and T4 polynucleotide kinase. 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 labeling 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 illustra ExoProStar, which includes both enzymes in a ready-to-use format.

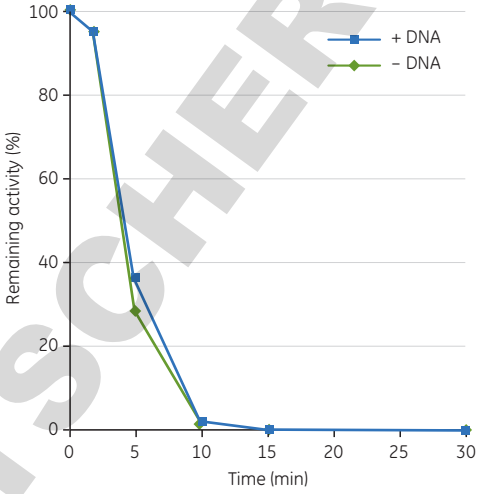

Fig 1. SAP inactivation at 65°C. Reactions were set up using 20 units SAP (excess) with or without 2 µg lambda DNA EcoRI/HindIII fragments. The reaction was incubated at 65°C to inactivate the SAP. Aliquots from the reaction were placed on ice at selected time intervals and assayed for activity in the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

Specifications

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

Functional Test: Dephosphorylation of a restriction enzyme digested plasmid (5–20 pmol of 5′ ends, 0.1–0.5 units/pmol 5′ ends). Reduces religation 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.

Ordering information

Product	Quantity	Code number
Shrimp Alkaline Phosphatase	500 units	E70092Y
Shrimp Alkaline Phosphatase	1000 units	E70092Z
Shrimp Alkaline Phosphatase	5000 units	E70092X
Exonuclease I	2500 units	E70073Z
Exonuclease I	5000 units	E70073X
ExoProStar S	100 reactions	US79010
ExoProStar S	500 reactions	US79050
ExoProStar S	2000 reactions	US79200
ExoProStar S	5000 reactions	US79500

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