

Phosphatase treatment of DNA fragments

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Reagents

- ◆ BAP (bacterial alkaline phosphatase)
- ◆ 10 × BAP buffer (500 mM Tris-HCl, pH 8.0, 10 mM ZnCl₂)
- ◆ Proteinase K (100 µg/µl)
- ◆ 10% SDS (sodium dodecyl sulphate) solution
- ◆ TE, pH 8.0 (see [Purification of DNA by phenol extraction and ethanol precipitation](#))
- ◆ CIP (calf intestinal phosphatase)
- ◆ 10× CIP buffer (200 mM Tris-HCl, pH 8.0, 10 mM MgCl₂, 10 mM ZnCl₂)
- ◆ Water-bath or heating-block

A. Phosphatase treatment with BAP

- 1 Prepare a reaction mixture containing appropriate amounts of 10× BAP buffer and DNA in a 100 µl volume. Check the enzyme supplier's documentation on enzyme activity.
- 2 Add 1 unit of BAP and incubate at 60 °C for 30 min. This high temperature suppresses any residual exonuclease activity present in the enzyme preparation and is recommended when using BAP.
- 3 Stop the reaction by adding SDS to a final concentration of 0.1% and proteinase K to 100 µg/µl. Incubate at 37 °C for 30 min.
- 4 Purify the phosphatase-treated DNA by phenol extraction and ethanol precipitation (see [Purification of DNA by phenol extraction and ethanol precipitation](#)) and resuspend the DNA in sterile TE, pH 8.0, or water.
- 5 Because of the stability of the enzyme and the consequences of its persistence in subsequent experiments, further purifications of the DNA are often performed before ethanol precipitation, e.g. include an extra phenol extraction.

B. Phosphatase treatment with CIP

- 1 Set up a reaction mixture as in step A.1 above with 10× CIP buffer.
- 2 Add 1 unit of CIP and incubate at 37 °C for 30 min.

- 3 Stop the reaction by heating the mixture at 75 °C for 10 min (CIP is heat-labile whereas BAP is not) before purifying the phosphatase-treated DNA by phenol extraction and ethanol precipitation (see [Purification of DNA by phenol extraction and ethanol precipitation](#)).

Phenol is a hazardous organic solvent. Always use suitable laboratory gloves when handling phenol containing solutions. Specific waste procedures may be required for the disposal of phenol containing solutions.

Notes

- (a) Shrimp alkaline phosphatase is also available from commercial suppliers and, as it is more heat-labile than CIP, is often used when absolutely no carry-over of inactivated phosphatase into later experimental manipulations can be tolerated. Use with a 10× buffer comprising 20 mM Tris-HCl, pH 8.0, 100 mM MgCl₂ and stop the reaction by heating at 65 °C for 15 min.
- (b) The efficiency of phosphatase treatment of DNA termini can be assessed by performing simple ligation reactions (see [DNA ligation](#)) in comparison with controls containing non-phosphatased DNA. The controls should show the ability to ligate, while correctly phosphatased DNA fragments should not.