

Dephosphorylation of Plasmid DNA

During ligation in vitro, T4 DNA ligase will catalyze the formation of a phosphodiester bond between adjacent nucleotides only if one nucleotide carries a 5'-phosphate residue and the other carries a 3'-hydroxyl terminus. Recircularization of vector DNA can therefore be minimized by removing the 5'-phosphate residues from both termini of the linear, double-stranded plasmid DNA with alkaline phosphatase.

Buffers and Solutions

EDTA (0.5 M, pH 8.0) or EGTA (0.5 M, pH 8.0)

SDS (10% w/v)

Sodium acetate (3 M, pH 5.2 and pH 7.0)

TE (pH 8.0)

Tris-Cl (10 mM, pH 8.3)

Proteinase K (10 mg/ml)

1. Digest a reasonable quantity of closed circular plasmid DNA (10 µg) with a two- to threefold excess of the desired restriction enzyme for 1 hour.
2. Remove an aliquot (0.1 µg), and analyze the extent of digestion by electrophoresis through a 0.7% agarose gel containing ethidium bromide, using undigested plasmid DNA as a marker. If digestion is not complete, add more restriction enzyme and continue the incubation.
3. When digestion is complete, extract the sample once with phenol:chloroform and recover the DNA by precipitation with ethanol.
4. Dissolve the DNA in 100 µl of 10 mM Tris-Cl (pH 8.3). (Remove 10 µl for testing of dephosphorylation efficiency)
5. To the remaining 90 µl of the linearized plasmid DNA, add 10 µl of 10x CIP or 10x SAP buffer and the appropriate amount of calf intestinal phosphatase (CIP) or shrimp alkaline phosphatase (SAP) and incubate as described in the table below:

Conditions for Dephosphorylation of 5'-phosphate Residues from DNA

Type of Terminus	Enzyme/Amount per mole DNA Ends	Incubation Temperature/Time
5'-Protruding	0.01 unit CIP ^a	37°C/30 minutes
	0.1 unit SAP	37°C/60 minutes
3'-Protruding	0.1-0.5 unit CIP ^b	37°C/15 minutes <i>then</i> 55°C/45 minutes
	0.5 unit SAP	37°C/60 minutes
Blunt	0.1-0.5 unit CIP ^b	37°C/15 minutes <i>then</i> 55°C/45 minutes
	0.2 unit SAP	37°C/60 minutes

a After the initial 30-minute incubation, add a second aliquot of CIP enzyme and continue incubation for another 30 minutes at 37°C.

b Add a second aliquot of CIP just before beginning the incubation at 55°C.

6. Inactivate the phosphatase activity:

To inactivate CIP at the end of the incubation period: Add SDS and EDTA (pH 8.0) to final concentrations of 0.5% and 5 mM, respectively. Mix well, and add proteinase K to a final concentration of 100 µg/ml. Incubate for 30 minutes at 55°C.

*Alternatively, CIP can be inactivated by heating to 65°C for 30 minutes (or 75°C for 10 minutes) in the presence of 5 mM EDTA or 10 mM EGTA (both at pH 8.0).
or*

To inactivate SAP: Incubate the reaction mixture for 15 minutes at 65°C in the dephosphorylation buffer.

7. Cool the reaction mixture to room temperature, and then extract it once with phenol and once with phenol:chloroform.
8. Recover the DNA by precipitation with ethanol.
9. Wash the pellet with 70% ethanol at 4°C.
10. Carefully remove the supernatant and leave the open tube on the bench to allow the ethanol to evaporate.
11. Dissolve the precipitated DNA in TE (pH 8.0) at a concentration of 100 ng/µl.