Dephosphorylation of 5´-ends of DNA using CIP (M0290)

Version 2

New England Biolabs

Abstract

Protocol for Dephosphorylation of 5´-ends of DNA using CIP in Restriction Enzyme Reaction. Uses the Calf Intestinal Alkaline Phosphatase (CIP - M0290)

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Guidelines

Dephosphorylation of 5' -ends of DNA in Restriction Enzyme Reaction

- The phosphate can be added directly into the digestion reaction during or after DNA digestion
- CIP is active in all NEB restriction enzyme buffers
- DNA purification is required before ligation

Materials

Alkaline Phosphatase, Calf Intestinal (CIP) M0290 by New England Biolabs

Protocol

Step 1.

Prepare a 20 µl reaction as follows:

DNA	1 pmol of DNA ends*
CutSmart® Buffer (10X)	2 µl
CIP	1 unit
H_2O, purified	to 20 µl**

NOTES
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** Note: 1 pmol of DNA ends is about 1 µg of a 3 kb plasmid.*

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** *Scale larger reaction volumes proportionally.*

Step 2.

Incubate at 37°C for 30 minutes.

DURATION
01:00:00

Step 3.

Purify DNA by gel purification, spin-column or phenol extraction.