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Feb 21, 2022

Dephosphorylation of 5´-ends of DNA using CIP (M0290) V.3

New England Biolabs[1]

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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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https://www.neb.com/protocols/0001/01/01/protocol-for-dephosphorylating-with-cip

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Dephosphorylation, CIP, Calf intestine phosphatase, DNA

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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) **New England Biolabs Catalog #M0290**

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

1 

Prepare a **20 µL** reaction as follows:

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

** Note: 1 pmol of DNA ends is about 1 µg of a 3 kb plasmid.*
*** Scale larger reaction volumes proportionally.*

2 

Incubate at **37 °C** for **00:30:00** .

3 Purify DNA by gel purification, spin-column (NEB #T1020 or NEB #T1030) or phenol extraction.

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