Dephosphorylation using CIP in Restriction Enzyme Reaction (M0290)

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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Materials

Alkaline Phosphatase, Calf Intest (CIP) - 1,000 units M0290S by New England Biolabs

Protocol

Step 1.
Digest 1–5 µg of plasmid DNA in a **20 µl** reaction as follows:

PROTOCOL

. Mixture for M0290 CIP

CONTACT: New England Biolabs

ANNOTATIONS
New England Biolabs 25 Jan 2015
Scale larger reaction volumes proportionally.

Step 1.1.
DNA ≥ **1 µl**

ANNOTATIONS
Peter Rüthemann 14 Oct 2015
Cas9

Step 1.2.
Restriction Enzyme Buffer (10X) **2 µl**

Step 1.3.
Restriction Endonuclease **1 µl**

Step 1.4.
H2O, purified to **20 µl**

Step 2.

Incubate at 37°C for 60 minutes or follow manufacturer’s recommendations.

DURATION

01:00:00

Step 3.

Add 1 unit of CIP for every 1 pmol of DNA ends (about 1 µg of a 3 kb plasmid).

ANNOTATIONS

Mohd Fahrurrazi Tompang 25 Apr 2018

How much exactly 1 unit of CIP in uliter

Step 4.

Incubate at 37°C for 30–60 minutes.

DURATION

01:00:00

Step 5.

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

Step 6.

Proceed with ligation.