Protocol for Dephosphorylation of 5′ ends of DNA using Quick CIP (NEB #M0525)

Overview

1. Prepare a 20 µl reaction as follows:

DNA	1 pmol of DNA ends*
rCutSmart™ Buffer (10X)	2 µl
Quick CIP	1 µl
Nuclease-free water	to 20 µl**

2. Incubate at 37°C for 10 minutes.

3. Stop reaction by heat-inactivation at 80°C for 2 minutes.

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

*** Scale larger reaction volumes proportionally.*

Dephosphorylation of DNA 5′-ends using Quick CIP in a Restriction Enzyme Reaction

- The phosphatase can be added directly into the digestion reaction during or after DNA digestion
- Add 1 µl of Quick CIP for every 1 pmol of DNA ends (about 1 µg of a 3 kb plasmid) and incubate at 37°C for 10 minutes
- Quick CIP is active in all NEB restriction enzyme buffers
- The restriction enzyme should be heat inactivated at the same time as the phosphatase after digest and dephosphorylation
- If restriction enzyme(s) cannot be heat inactivated, DNA purification is required before ligation