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Protocol for Dephosphorylation of 5′ ends of DNA using Quick CIP (NEB #M0525) V.1

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Protocol status: Working
We use this protocol and it's working

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Keywords: phosphatase, intestinal alkaline phosphatase, need for multiple phosphatase, multiple phosphatase, protocol for dephosphorylation, phosphates from dna, snp analysis recombinant for purity, snp analysis recombinant, native enzyme faster reaction setup, active in any restriction enzyme buffer, restriction enzyme buffer, using quick cip, dephosphorylation, less enzyme, enzyme, shorter incubation time flexible reaction condition, dna sequencing, phosphate, quick cip

Abstract

Quick CIP is a heat-labile version of calf intestinal alkaline phosphatase (CIP) purified from a recombinant source.

- Rapid and irreversible heat inactivation eliminates unwanted activity
- Improved storage stability versus native enzyme
- Faster reaction setup (no supplemental additives like zinc required) and shorter incubation time
- Flexible reaction conditions (active in any restriction enzyme buffer, no clean-up required)
- Less enzyme required (high specific activity), resulting in a lower cost per reaction
- No need for multiple phosphatases (Quick CIP removes 5′- and 3′- phosphates from DNA, RNA and dNTPs)
- Active on unincorporated dNTPs in PCR products - improves DNA sequencing and SNP analysis
- Recombinant for purity, consistency and value

Materials

MATERIALS

Quick CIP **New England Biolabs Catalog #**M0525

CutSmart® Buffer **New England Biolabs Catalog #**B7204S

Safety warnings

Please see SDS (Safety Data Sheet) for hazards and safety warnings.

Before start

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

1 Prepare a 20 µL reaction as follows:

	DNA	1 pmol of DNA ends *
	CutSmart® Buffer (10X)	2 µl
	Quick CIP	1 µl
	H2O, 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 00:10:00 .

3 Stop reaction by heat-inactivation at 80 °C for 00:02:00 .