MAY 16, 2020

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.7r2hm8e

External link:
<https://neb.com/protocols/2019/06/04/protocol-for-dephosphorylation-of-5-ends-of-dna-using-quick-cip-neb-m0525>

Protocol Citation: New England Biolabs 2020. Protocol for Dephosphorylation of 5' ends of DNA using Quick CIP (NEB #M0525).
protocols.io
<https://dx.doi.org/10.17504/protocols.io.7r2hm8e>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: Sep 27, 2019

Last Modified: Mar 29, 2021

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

New England Biolabs¹

¹New England Biolabs

New England Biolabs (NEB)

Tech. support phone: +1(800)632-7799 email: info@neb.com

New England Biolabs

New England Biolabs

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.

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

Keywords: phosphatase

- 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 .