May 16, 2020

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

New England Biolabs[1]

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1 Works for me dx.doi.org/10.17504/protocols.io.7r2hm8e

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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

EXTERNAL LINK

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

MATERIALS

| NAME | CATALOG # | VENDOR |
|---|---|---|
| Quick CIP | M0525 | New England Biolabs |
| CutSmart® Buffer | B7204S | New England Biolabs |

SAFETY WARNINGS

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

BEFORE STARTING

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:



**Citation:** New England Biolabs (05/16/2020). Protocol for Dephosphorylation of 5Ã¢ÂÂ² ends of DNA using Quick CIP (NEB #M0525). https://dx.doi.org/10.17504/protocols.io.7r2hm8e

| | |
|---|---|
| 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** .



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