

# Non-radioactive phosphorylation with T4 PNK 3' phosphatase minus

New England Biolabs

## Abstract

Protocol for non-radioactive phosphorylation with T4 PNK 3' phosphatase minus.

**Citation:** New England Biolabs Non-radioactive phosphorylation with T4 PNK 3' phosphatase minus. **protocols.io**  
dx.doi.org/10.17504/protocols.io.cp2vqd

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## Materials

 T4 Polynucleotide Kinase (3' phosphatase minus) - 200 units [M0236S](#) by [New England Biolabs](#)

## Protocol

### Step 1.

Set up the following reaction.

#### [PROTOCOL](#)

#### [. T4 PNK Minus Reaction](#)

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#### [NOTES](#)

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1X T4 DNA Ligase Buffer contains 1 mM ATP and can be substituted in non-radioactive phosphorylations (T4 Polynucleotide Kinase exhibits 100% activity in this buffer).

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ATP is not supplied

#### **Step 1.1.**

DNA up to 300pmol of 5' termini

#### **Step 1.2.**

10X T4PNK Reaction Buffer, **5 µl**

 [AMOUNT](#)

5 µl Additional info:

#### **Step 1.3.**

10mM ATP, **5 µl**

 [AMOUNT](#)

5 µl Additional info:

#### **Step 1.4.**

T4 PNK (3' phosphatase minus), **1 µl** (10 units)

 **AMOUNT**

1 µl Additional info:

**Step 1.5.**

Water up to **50 µl**

**Step 2.**

Incubate at 37°C for 30 minutes.

 **DURATION**

00:30:00

**Step 3.**

Heat inactivate by incubating at 65°C for 20 minutes.

 **DURATION**

00:20:00