

2 ▼

Apr 12, 2022

Non-radioactive phosphorylation with T4 PNK 3' phosphatase minus (M0236) V.2

New England Biolabs¹¹New England Biolabs

1

[dx.doi.org/10.17504/protocols.io.4rm7vzdr1w/v2](https://doi.org/10.17504/protocols.io.4rm7vzdr1w/v2)**New England Biolabs (NEB)**Tech. support phone: **+1(800)632-7799** email: **info@neb.com****New England Biolabs**
New England Biolabs

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

DOI

[dx.doi.org/10.17504/protocols.io.4rm7vzdr1w/v2](https://doi.org/10.17504/protocols.io.4rm7vzdr1w/v2)<https://www.neb.com/protocols/0001/01/01/non-radioactive-phosphorylation-with-t4-pnk-or-pnk3-phosphatase-minus>

New England Biolabs 2022. Non-radioactive phosphorylation with T4 PNK 3' phosphatase minus (M0236). **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.4rm7vzdr1w/v2>

New England Biolabs



phosphorylating, T4PNK reaction buffer, PNK, T4 PNK 3' phosphatase

_____ protocol ,

May 25, 2020

Apr 12, 2022


37461

MATERIALS

[Adenosine-5 Triphosphate \(ATP\) - 1 ml](#) **New England****Biolabs Catalog #P0756S**[T4 Polynucleotide Kinase \(3' phosphatase minus\) - 200 units](#) **New England****Biolabs Catalog #M0236S**

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

1 

Set up the following reaction in a microcentrifuge tube  **On ice** :



A	B
Reagent	Volume
DNA	up to 300 pmol of 5' termini
T4 PNK Reaction Buffer (10X)	5 µl
ATP (10 mM)	5 µl
T4 PNK	1 µl (10 units)
Nuclease-free Water	up to 50 µl

- ATP is not supplied
- 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).

2 

Incubate at  **37 °C** for  **00:30:00** .

3 

Heat inactivate by incubating at  **65 °C** for  **00:20:00** .