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# Non-radioactive phosphorylation with T4 PNK (M0201) V.2

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[dx.doi.org/10.17504/protocols.io.bdd2i28e](https://dx.doi.org/10.17504/protocols.io.bdd2i28e)**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.

DOI

[dx.doi.org/10.17504/protocols.io.bdd2i28e](https://dx.doi.org/10.17504/protocols.io.bdd2i28e)<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 (M0201). **protocols.io**<https://dx.doi.org/10.17504/protocols.io.bdd2i28e>

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phosphorylating, T4PNK reaction buffer, PNK, T4 PNK 3' phosphatase, M0201

protocol ,

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## MATERIALS

 Adenosine-5 Triphosphate (ATP) - 1 ml **New England**

**Biolabs Catalog #P0756S**


 T4 Polynucleotide Kinase - 500 units **New England**

**Biolabs Catalog #M0201S**

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** .