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3' Azide labelling with TdT

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ABSTRACT

Protocol for add azide modified nucleotide to 3' end of unmodified DNA oligonucleotide using terminal transferase. Protocol adapted from [Winz et al. 2015](#).

MATERIALS

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✂ Terminal Transferase - 2,500 units **New England Biolabs Catalog #M0315L**

✂ ethanol **Contributed by users**

✂ N6-(6-Azido)hexyn-3dATP **Jena Bioscience Catalog #NU-1707**

✂ 3M Sodium Acetate solution **Contributed by users**

OPEN ACCESS

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Protocol status: In development
We are still developing and optimizing this protocol

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PROTOCOL integer ID:
12717

TdT reaction

1 Set up TdT reaction

- 2.5 µL 10x Terminal transferase buffer
- 10 µL 2.5mM CoCl₂
- 2 µL 400µM template oligo
- 1 µL 10mM N6(6-azido)hexyl-3'-dATP
- 2.5 µL recombinant TdT (20U/µL)
- 7 µL nuclease free water

Note

Probably useful to scale up reaction.

- 2 Incubate at 37°C overnight.

- 37 °C Incubate on thermal cycler
- 50 °C thermal cycler lid temperature
- 12:00:00 incubate overnight

Ethanol precipitate to cleanup

- 3 Add 1/10th volume sodium acetate and 3x volume ethanol to precipitate azide labelled oligo.

- 2.5 µL 3M sodium acetate
- 82.5 µL 200 proof ethanol

Note

If you want to get rid of excess N6-(6-azido)hexyl-3'-dATP, you may want to use 1/2 volume 7.5M ammonium acetate.

- 4 Place solution at -80°C for 1 hour to precipitate.

Note

Fine to incubate at -20°C or -80°C for longer.

- 80 °C
- 01:00:00

- 5 Centrifuge for 10 minutes to precipitate

- 00:10:00 Centrifuge at 16,000 x g
- 4 °C

- 6 Remove supernatant. Careful not to disturb pellet.

7 Wash sample with 500 μ L 70% ethanol

🧪 500 μ L 70% ethanol

8 Centrifuge for 2-5 minutes.

🕒 00:02:00 Centrifuge at 16,000 x g

9 Remove supernatant. Careful not to disturb pellet.

10 Centrifuge for 1 minute to collect supernatant from the lid and walls.

🕒 00:01:00 Centrifuge at 16,000 x g

11 Remove supernatant then dry pellet under vacuum or ambient air.

12 Add 1.8 μ L nuclease free water to dissolve dry pellet. Let sit at room temp for ≥ 20 minutes.

🧪 1.8 μ L nuclease free water

🌡️ 25 °C incubate at room temperature

🕒 00:20:00 incubate at room temperature.

Note

Adding 1.8 μ L and assuming ~10% loss. Can be more precise and measure final concentration but beware of residual dATP.

13 Store azide modified oligo at -20°C indefinitely.

🌡️ -20 °C