3' Azide labelling with TdT

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Abstract

Protocol for add azide modified nucleotide to 3' end of unmodified DNA olgionucleotide using terminal transferase. Protocol adapted from Winz et al. 2015.

Citation: Benjamin Emert 3' Azide labelling with TdT. **protocols.io**
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Materials

- Terminal Transferase - 2,500 units M0315L by New England Biolabs
- ethanol by Contributed by users
  N6-(6-Azido)hexyn-3dATP NU-1707 by Jena Bioscience
- 3M Sodium Acetate solution by Contributed by users

Protocol

TdT reaction

Step 1.

Set up TdT reaction

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

NOTES
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Probably useful to scale up reaction.

TdT reaction

Step 2.

Incubate at 37ºC overnight.

TEMPERATURE
37 °C Additional info: Incubate on thermal cycler
TEMPERATURE
50 °C Additional info: thermal cycler lid temperature

Ethanol precipitate to cleanup

Step 3.

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

AMOUNT
2.5 µl Additional info: 3M sodium acetate
AMOUNT
82.5 µl Additional info: 200 proof ethanol

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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.

Ethanol precipitate to cleanup

Step 4.

Place solution at -80ºC for 1 hour to precipitate.

TEMPERATURE
-80 °C Additional info:

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Fine to incubate at -20ºC or -80ºC for longer.

Ethanol precipitate to cleanup

Step 5.

Centrifuge for 10 minutes to precpitate

TEMPERATURE
4 °C Additional info:

Ethanol precipitate to cleanup

Step 6.

Remove supernatant. Careful not to disturb pellet.

Ethanol precipitate to cleanup

Step 7.

Wash sample with 500 µL 70% ethanol

AMOUNT
500 µl Additional info: 70% ethanol

Ethanol precipitate to cleanup
Step 8.

Centrifuge for 2-5 minutes.

Ethanol precipitate to cleanup
Step 9.

Remove supernatant. Careful not to disturb pellet.

Ethanol precipitate to cleanup
Step 10.

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

Ethanol precipitate to cleanup
Step 11.

Remove supernatant then dry pellet under vacuum or ambient air.

Ethanol precipitate to cleanup
Step 12.

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

AMOUNT
1.8 µl Additional info: nuclease free water
TEMPERATURE
25 °C Additional info: incubate at room temperature

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

Ethanol precipitate to cleanup
Step 13.

Store azide modified oligo at -20ºC indefinitely.

TEMPERATURE
-20 °C Additional info: