

Removal of genomic DNA from RNA preparations (Thermo Scientific) Version 2

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Abstract

Removal of genomic DNA from RNA preparations

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Guidelines

allways waer gloves and work on ice

Protocol

Step 1.

Add to an RNase free tube:

RNA	1 µg
10X reaction buffer with MgCl ₂	1 µl
DNase I, RNase-free	1 µl (1U)
Water	to 10 µl

Step 2.

Incubate at 37 °C for 30 min

🌡 TEMPERATURE

37 °C Additional info:

Step 3.

Add EDTA, Water and PCI and vortex thoroughly.

📄 AMOUNT

1 µl Additional info: EDTA

📄 AMOUNT

80 µl Additional info: Water

AMOUNT

100 µl Additional info: PCI (phenol chloroform isoamyl alcohol)

Step 4.

Centrifuge for 10 min at 10000 rpm and 4 °C

Step 5.

transfer the upper phase into a fresh tube and add 3 volumen EtOH/ 3M Natrumacetat (30:1, ph 5.2)

Step 6.

precipitate RNA over night at -20 °C

Step 7.

Centrifuge 30 min at 13000 rpm and 4 °C

TEMPERATURE

4 °C Additional info:

Step 8.

Discard supernatant and wash pellet with 75% EtOH (do not resuspend the pellet)

Step 9.

Centrifuge 10 min at 13000 rpm and 4 °C

TEMPERATURE

4 °C Additional info:

Step 10.

GOTO

Centrifuge -> go to step #9

Step 11.

Discard supernatant and dry pellet for 10 - 15 min

Step 12.

resuspend pellet with 30 µl H₂O