Mar 22, 2019

Removal of genomic DNA from RNA preparations (Thermo Scientific)- (M4455 Version)

Forked from Removal of genomic DNA from RNA preparations (Thermo Scientific)

DOI
dx.doi.org/10.17504/protocols.io.ze2f3ge

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DOI: dx.doi.org/10.17504/protocols.io.ze2f3ge

Protocol Citation: Maximilian Dietsch 2019. Removal of genomic DNA from RNA preparations (Thermo Scientific)- (M4455 Version). **protocols.io https://dx.doi.org/10.17504/protocols.io.ze2f3ge**

Protocol status: Working
We use this protocol and it's working

Created: March 22, 2019

Last Modified: March 22, 2019

Protocol Integer ID: 21690

Abstract

Removal of genomic DNA from RNA preparations

Guidelines

allways waer gloves and work on ice

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

2 Incubate at 37 °C for 30 min

37 °C

00:30:00

3 Add EDTA, Water and PCI and vortex thoroughly.

1 µL EDTA

80 µL Water

100 µL PCI (phenol chloroform isoamyl alcohol)

4 Centrifuge for 10 min at 10000 rpm and 4 °C

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

6 precipitate RNA over night at -20 °C

7 Centrifuge 30 min at 13000 rpm and 4 ° C

4 °C

00:30:00

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

9 Centrifuge 10 min at 13000 rpm and 4 °C

4 °C

00:10:00

10 go to step #8 repeat washing step

go to step #9 Centrifuge

11 Discard supernatant and dry pellet for 10 - 15 min

12 resupend pellet with 30 µl H_20