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Removal of gDNA from totalRNA

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1 Works for me dx.doi.org/10.17504/protocols.io.8bfhsjn

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ABSTRACT

Removal of genomic DNA from RNA preparations

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0012000_DNase_I_RNasefree_1UuL_UG.pdf

GUIDELINES

Always work with gloves and safety gear and work on ice.

MATERIALS TEXT

- RNase-free H_2O
- DNaseI-buffer (10X)
- DNaseI
- 50 mM EDTA
- NaOAc (3M & pH 5,3) and 100% EtOH

Digestion of DNA

1 Calculate the volume required for 1 µg RNA

Component	amount
DNaseI buffer	1 µl
total RNA	1 µg
DNaseI	1 µl
H2O	to 10 µl

2 pipet components into a RNase-free 1.5 ml tube (following order: H2O, buffer, total RNA, DNaseI)

3 incubate reaction at 37°C

00:30:00

Extraction of DNaseI-digested RNA

4 add 1 µl 50 mM EDTA and incubate at 65°C

00:10:00

5 add 1/10 Volume NaOAc and 3 Volumes 100% EtOH

6 freeze at -20°C over night or at -80°C for at least 1h

7 centrifuge at 13000 rpm at 4°C

00:30:00

8 Discard supernatant

RNA pellet may be invisible, make sure to align tube lid so you remember where the pellet is located

9 add 500 µl 70% EtOH

10 centrifuge at 13000 rpm at 4°C

00:10:00

11 discard supernatant, remove as much ethanol as possible

12 dry tubes at RT under the hood (~5min), do not overdry!

13 resuspend pellet in 20 µl RNase-free water

14 measure concentration

Quality control:

15 perform PCR on your DNA-free RNA. Include a positive control using gDNA as a template and primer that bind to it.

There should be no visible bands except for positive control!