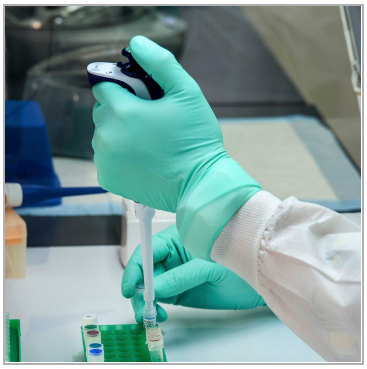

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We use this protocol and it's working

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gDNA RNA Clean Up Protocol

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ABSTRACT

Protocol used to eliminate DNA fragments and gDNA from purified RNA and to remove impurities from the sample.

GUIDELINES

Important considerations:

***Multiplying by 1.06 for each reagent in solution preparation is required due to pipetting error.

***Before starting read the protocol and ensure that the required volume of each reagent is available.

75% ethanol should be prepared with ***NEW DEPC H2O and Molecular Biology Grade (200 Proof >99.45%) Ethanol.

***Prepare all solutions in advance or check that all solutions are available.

***This protocol is to be used only with DNAse I M0303 from NEB. When using with another DNAse I, adjust the reaction volumes and adjust the final volume of the RNA precipitation mix to 700 uL.

MATERIALS

- DNAse I (M0303S);
- 10X DNAse I Buffer;
- Milli-Q water treated with 0.1% DEPC;
- 4M ammonium acetate ($NH_4CH_3CO_2$) - Must be filtered through a 0.22um filter;
- RNAse Free 0.5M EDTA pH 8.0 - Must be filtered through a 0.22um filter;
- Ethanol 75% - Molecular Biology 200 proof;
- New 50 mL Falcon tubes;
- Pipettes: P1000, P200, P10;
- Pipette Tips: 1000, 200, 10 uL;
- Thermomixer (37 ºC);
- Refrigerated centrifuge (4°C);
- Ice;
- Styrofoam Box;
- Rack for 1.5 mL microtubes;
- Exhaust Chapel;
- Electrophoresis vat;
- Agarose;
- TAE 1X;
- Nanodrop, Microdrop or Qubit;

SAFETY WARNINGS

***BOOK ALL EQUIPMENT IN ADVANCE AND CHECK THAT ALL MATERIAL WILL BE AVAILABLE.
***KEEP SAMPLES ON ICE DURING ALL HANDLING, AND FREEZE AT -80ºC AFTER USE.

BEFORE START INSTRUCTIONS

*** Use only sterile RNAse-Free tubes - AM12425 - or sterile Axygen RNAse Free microtubes.
*** All calculations should be performed before the beginning of the procedure, since reagents and samples have high added value.

Procedure

1 Starting with the 27 uL left over from each sample, add another 62 uL of RNAse-Free H2O at room temperature to all samples; 5m

2 Prepare DNAse Mix I and add 11 uL of the Mix per sample; 15m

10 uL of 10X DNAse I Buffer * No. of samples;

1 uL of DNAse I * No. of samples;

—

11 uL * No. of samples * 1.06

*** Final reaction volume = 100 uL, **adjust the final volume if less than 100 uL.**

3 Incubate the samples in the thermomixer at 37º C, without shaking, for 20 minutes; 20m

4 Add 1 uL of RNAse Free 0.5M EDTA pH 8.0 to each sample; 20m

5 Incubate at 75°C for 10 minutes; 10m

6 Prepare the **RNA Precipitation Mix:** 5m

150 uL Water treated with 0.1% DEPC * No. of samples

100 uL 4M Ammonium Acetate * No. of samples

350 uL Ethanol 200 proof Molecular Biology Grade (99.45%) * No. of samples

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600 uL of Mix * No. of samples * 1.06

7 Add 600 uL of the RNA Precipitation Mix to each sample and **INVERT** 20X using a microtube rack; 3m

8 Incubate at -80°C for 30 minutes or overnight at -20°C; 12h

9 Centrifuge at 4°C for 20 minutes at 13,000 RPM; 20m

10 Discard the supernatant with the aid of pipette tips; 10m

11 Add 300 uL of 75% Molecular Biology Grade Ethanol prepared with 0.1% DEPC-treated Milli-Q Water (only add to wash the pellet, and vortex for less than 1 second); 5m

12 Centrifuge at 4°C for 5 minutes at 13,000 RPM; 5m

13 Discard the supernatant with the aid of pipette tips; 10m

14 Add 300 uL of 75% Ethanol for Molecular Biology prepared with 0.1% DEPC-treated Milli-Q Water (only add to wash the pellet, and vortex for less than 1 second); 5m

15 Centrifuge at 4°C for 7 minutes at 13,000 RPM; 7m

16 Discard the supernatant with the aid of pipette tips; 10m

17 Leave the tubes open in a 1.5 mL microtube rack in the fume hood for 5 minutes; 5m

18 Resuspend the pellet in 20 uL of 0.1% DEPC-treated Milli-Q Water at 60 °C from tube to tube, homogenizing moderately for 30 seconds per sample (use P20 in 15 uL volume); 30m

19 Run 1.5% agarose gel in 1X TAE buffer + 5% bleach (80 V - 80 min); 1h 40m

***The electrophoresis vat should be thoroughly cleaned with RNAse Zap + Distilled Water and fresh running buffer should be added, the gel polymerization tray should be thoroughly cleaned and the gel should be made with fresh buffer to avoid RNA degradation.

19.1 If the RNA is intact (identify 18S and 28S rRNA bands at 2Kb and 4.8Kb), determine the concentration and quality parameters (A260/A230 and A260/A280) of the sample in the spectrophotometer/nanodrop;

20 If RNA is used in more sensitive applications, quantify in Qubit;

21 Store the samples in the ultra-freezer (-80 ºC) for up to 6 months.