Purification of RNA from Crude NA Extract

The following protocol is intended for the purification of DNA-free RNA from a crude extract of nucleic acids.

Reagents and kits

1. DNase I (preferably Ambion's TURBO DNase).
2. Qiagen's RNeasy MinElute Cleanup.
3. Optional: RNase inhibitor (Invitrogen's RNaseOUT™, Promega's RNasin™, or Fermentas' Ribolock™).
4. Optional: Ambion's RNA Storage Solution.

Before you begin

1. Optional: add 10 µL β-ME or 20 µL 2M DTT per 1 mL buffer RLT. Store up to 1 month at RT.
2. For each sample prepare in a rack: 1 MinElute spin column, 2 collection tubes, 1 1.5 mL tube and mark them.
3. Dilute buffer RPE with 44 mL of 96-100% EtOH.

Procedure

1. a. If using Qiagen's DNase I or Ambion's TURBO DNase prepare the following mixture in a 1.5 mL tube:

NA crude extract	10-85.5 µL
10X Buffer	10 µL
RNase inhibitor (40 U/µL)*	2 µL
0.1 M DTT*	1 µL
DNase I	1 µL per 2 µg of DNA or 2.5 µL for Qiagen's DNase I
RNase free water	To 100 µL

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b. If using Fermantas' DNase I prepare the following mixture in a 1.5 mL tube:

NA crude extract	10-86 µL (or up to 1 µg per unit)
10X reaction buffer without $MgCl_2$	10 µL
$MnCl_2$ (100 mM)	1 µL
RNase inhibitor (40 U/µL)*	2 µL
0.1 M DTT*	1 µL
DNase I (1 U/µL)	1 µL per 1 µg of DNA
RNase free water	To 100 µL

* optional

2. Incubate at 37° C (room temperature for Qiagen's DNaseI) for 30 min.
3. Add 350 µL buffer RLT (per 100 µL digested sample) and mix well.
4. Add 250 µL 96-100% EtOH (per 100 µL digested sample) and mix well by pipetting.
5. Transfer immediately up to 700 µL of sample to the spin column, close the lid, and centrifuge for 15 s at ≥8000 × g (≥10000 rpm).
6. If sample volume is larger than 700 µL discard the flow through after centrifugation and repeat the process until all the sample has been passed through the column.
7. Place the spin column in a new collection tube and add 500 µL of buffer RPE, close the lid, and centrifuge for 15 s at ≥8000 × g (≥10000 rpm).
8. Discard the flow through and place spin column in the same collection tube.
9. Add 500 µL of 80% EtOH, close the lid, and centrifuge for 2 min at ≥8000 × g (≥10000 rpm).
10. Discard the flow through and place spin column in a new collection tube.
11. Open the lid, and centrifuge for 5 min at full speed.
12. Place the spin column in a 1.5 mL tube, add 20 µL (10-30 µL) of RNase free water or RNase Storage Solution, and centrifuge for 1 min at full speed.

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