Purification of DNA from Crude NA Extract

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

Reagents and kits

1. RNase ONE™ Ribonuclease (Promega).
2. Wizard® DNA Clean-Up System (Promega).
3. Isopropanol.

Before you begin

1. For each sample prepare in a rack: 1 – MinElute spin column, 2 – collection tubes, 1 – 1.5 ml tube and mark them.

Procedure

1. Incubate 10-100 µl of crude NA extract (up to 10 µg of total RNA at 85 °C for 5 min to denature the RNAs).
2. Prepare the following mixture in a 1.5 ml tube:

NA crude extract	50-89 µl (up to 20 µg)*
RNase digestion buffer	10 µl
RNase ONE™ Ribonuclease	1 µl
RNase free water	To 100 µl

* If sample volume is less than 50 µl, bring to 50 µl with DDW.

3. Incubate the samples for 60 min at 37 °C.
4. Attach one Syringe Barrel to a Minicolumn, and insert the tip to the vacuum manifold.
5. Shake well the Wizard® DNA Clean-Up Resin and add 1 ml to the sample tube and mix by inversion.
6. Pipette the resin/ sample mix into the Syringe Barrel.
7. Apply vacuum until solution is completely drawn.
8. Add 2 ml 80% isopropanol, and re-apply vacuum until solution is completely drawn.

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9. Continue applying the vacuum for extra 30 s after the solution has been drawn.
10. Remove the Syringe Barrel and transfer the minicolumn to a 1.5 ml tube, and centrifuge for 2 min at maximum speed.
11. Transfer the minicolumn to a new 1.5 ml tube, apply 50 µl of 70 °C water or low TE buffer and incubate 1 min.
12. Centrifuge the minicolumn at maximum speed for 30 s.
13. Run 2-5 µl on agarose gel to validate removal of RNA.
14. Quantify the DNA using a spectrophotometer or PicoGreen.

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