User-Developed Protocol

RNA purification from EZ1® DNA Investigator® waste fractions

This protocol is adapted from the QIAGEN RNeasy® MinElute® Cleanup protocol and is intended for use as a guideline for the purification of total RNA from waste fractions obtained from a sample first purified using the EZ1 DNA Investigator protocol.

This protocol has not been thoroughly tested and optimized by QIAGEN.

IMPORTANT: Please read the "Safety Information" and "Important Notes" sections in the *RNeasy MinElute Cleanup Handbook* before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, please consult the appropriate Safety Data Sheets (SDSs), available from the product supplier. The RNeasy MinElute Cleanup Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

Equipment and reagents to be supplied by user

- RNeasy MinElute Cleanup Kit (cat. no. 74204)
- Collection tubes, 2 ml (cat. no.19201)
- Ethanol (80% and 96–100%; do not use denatured alcohol, which contains other substances such as methanol or methylethylketone)
- Sterile, RNase-free pipet tips
- Disposable gloves
- 1.5 ml or 2 ml microcentrifuge tubes
- Microcentrifuge (with rotor for 2 ml tubes) for centrifugation at 20–25ºC
- Kimwipes®

Important points before starting

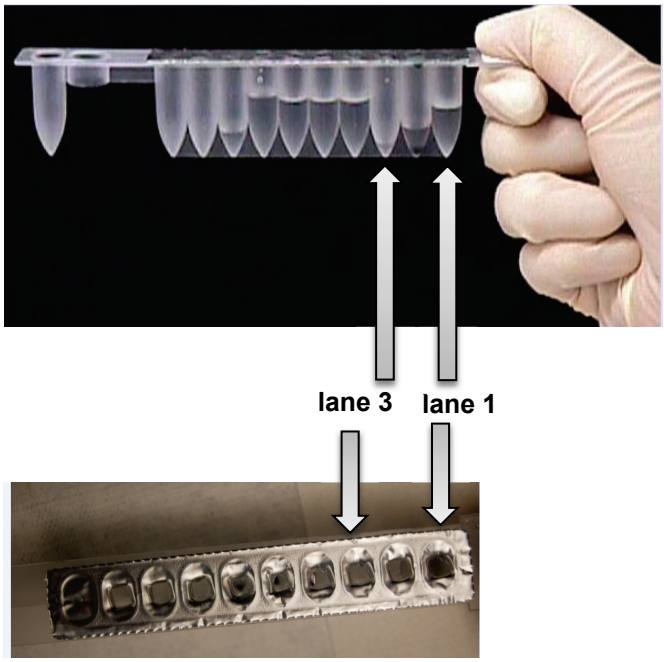
- If using RNeasy MinElute Cleanup Kits for the first time, read "Safety Information" (page 6) in the *RNeasy MinElute Cleanup Handbook*.
- If working with RNA for the first time, read Appendix A: General Remarks on Handling RNA (page 19) in the *RNeasy MinElute Cleanup Kit Handbook.*
- Buffer RLT contains guanidine thiocyanate and is therefore not compatible with disinfecting reagents containing bleach.
- Perform all steps of the procedure at room temperature (15–25ºC). During the procedure, work quickly.
- Ensure that the centrifuge does not cool below 20ºC.

Things to do before starting

- Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%) as indicated on the bottle to obtain a working solution.

- Buffer RLT may form a precipitate during storage. If necessary, redissolve by warming, and then place at room temperature (15–25ºC).
- Following completion of the EZ1 DNA Investigator extraction, transfer ~1 ml fraction from lane 1 (for Large-Volume Protocol) or lane 3 (for Trace Protocol) of the cartridge into an appropriately labeled snap-cap, 1.5 ml centrifuge tube. This waste fraction will be used for RNA purification.
- The waste fractions may be stored at –20ºC until needed, or at 4ºC for one week. The EZ1 waste fraction should be thawed at room temperature and vortexed to ensure homogeneity prior to use.

EZ1 DNA Investigator cartridge



Procedure

1. Transfer 200 µl of the thawed and well mixed EZ1 waste fraction into a 1.5 ml microcentrifuge tube.
2. Add 700 µl Buffer RLT and mix well by slowly pipetting up and down.
3. Add 500 µl 96–100% ethanol to the sample and mix well by slowly pipetting up and down.

   Important: Do not centrifuge.
4. Transfer a portion of the sample (700 µl) to an RNeasy MinElute spin column placed in a 2 ml collection tube. Close the lid gently, and centrifuge for 15 s at 11,000 rpm. Discard the collection tube containing the flow-through.
5. Place the spin column in a new 2 ml collection tube. Repeat step 4 with the remaining 700 µl of sample, and discard the collection tube containing the flow-through.
6. Place the spin column in a new 2 ml collection tube. Add 500 µl Buffer RPE to the spin column. Close the lid gently, and centrifuge for 15 s at 11,000 rpm. Discard the collection tube containing the flow-through.

7. Place the spin column in a new 2 ml collection tube. Add 500 µl of 80% ethanol to the spin column. Close the lid gently, and centrifuge for 2 min at 11,000 rpm to wash the spin column membrane. Discard the collection tube containing the flow-through.

   Note: After centrifugation, carefully remove the spin column from the collection tube so that the column does not contact the flow-through; otherwise, carryover of ethanol will occur.

8. Place the spin column in a new 2 ml collection tube. Open the lid of the spin column, and centrifuge at 11,000 rpm for 5 min.

   Note: To avoid damage to their lids, place the spin columns into the centrifuge with at least one empty position between columns. Orient the lids so that they point in a direction opposite to the rotation of the rotor (e.g., if the rotor rotates clockwise, orient the lids counterclockwise.

9. Place the spin column in a new 1.5 ml collection tube. Add 16 µl RNase-free water directly to the center of the spin column membrane. Close the lid gently, and centrifuge for 1 min at 11,000 rpm to elute the RNA.

10. Store the purified RNA at –20ºC until needed.

Reference

Hanson, E.K., Lopez, J., and Ballantyne, J. (2017) A novel DNA/RNA co-extraction method using EZ1 DNA Investigator extraction ‘waste’. ISFG, Seoul, Korea.