EMORY UNIVERSITY

Emory Integrated Genomics Core

Emory Integrated Core Facilities

EIGC.002_Appendix K_QIAGEN RNeasy Micro Kit for RNA Extraction from Tissue

Standard Operating Procedure Staff Review Page:

I have read and understand the procedure listed above.

Employee name	Date SOP review complete

Standard Operating Procedure Approval Page:

Date Implemented:	23 January 2008	
Updated:	Ashima Amin **Name**	15 June 2020 **Date**
Supersedes:	Ashima Amin **Name**	11 April 2016 **Date**

Annual Review and Approval

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Laboratory Director

Changes Made:
061520: Review, Protocols split out into appendixes. Renumbered SOPs. Previously EIGC.003.

Changes to previous procedures:
041116: Review, LIMS steps updated.

Introduction

The RNeasy Micro Kit uses a novel technology to purify RNA (maximum 45 µg) from small amounts of animal and human tissues (≤5 mg) or cells (as little as 1 cell and up to $5x10^5$), such as laser-microdissected (LMD) samples, fine- needle aspirates (FNA) and FACS® sorted cells. The main difference between the RNeasy and miRNeasy kits is the option in the latter to enrich for micro RNAs in the sample.

RNeasy Micro technology combines the selective binding properties of a silica-based membrane with the speed of microspin technology. Guanidine-thiocyanate–containing lysis buffer and ethanol are added to the sample to create conditions that promote selective binding of RNA to the RNeasy MinElute membrane. The sample is then applied to the RNeasy MinElute spin column. RNA binds to the silica membrane. Traces of DNA that may copurify are removed by DNase treatment on the RNeasy MinElute spin column. DNase and any contaminants are washed away, and high-quality total RNA is eluted in RNase-free water.

With the RNeasy Micro procedure, all RNA molecules longer than 200 nucleotides are purified. The procedure provides an enrichment for mRNA since most RNAs <200 nucleotides (such as 5.8S rRNA, 5S rRNA, and tRNAs, which together comprise 15–20% of total RNA) are selectively excluded. The size distribution of the purified RNA is comparable to that obtained by centrifugation through a CsCl cushion, where small RNAs do not sediment efficiently.

Kit Contents

Rneasy Micro Kit	**(50)**
Catalog no.	74004
Number of preps	50
RNeasy MinElute Spin Columns (each in a 2 ml Collection Tube)	50
Collection Tubes (1.5 ml)	50
Collection Tubes (2 ml)	100
Buffer RTL [a]	45 ml
Buffer RW1 [a]	45 ml
Buffer RPE (concentrate)[b]	11 ml
RNase-Free Water	3x10 ml
Carrier RNA, Poly A	310 µg
RNase-Free DNase Set:	
• **RNase-Free DNase I (lyophilized)**	1500 units
• **Buffer RDD**	2x2ml
• **RNase Free Water**	1.5 ml

[a] Contains guanidine salt. Not compatible with disinfecting agents containing bleach; see handbook for safety information.

[b] Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96%–100%)

Storage

The RNeasy Micro Kit (cat. no. 74004) is shipped at ambient temperature. Store the RNeasy MinElute® spin columns and the RNase-Free DNase Set immediately at 2–8°C. Store the remaining components dry at room

temperature (15–25°C). All kit components are stable for at 9 months after receiving under these conditions if not otherwise stated on label.

Procedure

Notes before starting

- Ensure that Buffer RPE has been prepared according to the instructions as indicated on the bottle or the table above.
- If purifying RNA from cell lines rich in RNases, add either 10 µl β-mercaptoethanol (β-ME), or 20 µl 2 M dithiothreitol (DTT) to 1 ml Buffer RLT Plus before use. Buffer RLT Plus containing -ME or DTT can be stored at room temperature for up to 1 month.
- Pre-heat RNase Free water at 70°C on the heat block. Pre-heated RNase Free water will be used just in elution step. Do not use pre-heated water to make any solution.
- Prepare DNase solution
    - To make the DNase Stock Solution, use a syringe and needle to add 550 µl of water (provided in the DNase kit) to the vial of lyophilized DNase. Mix gently. Remove the stopper and carefully pipet 65 µL aliquots of the stock solution into 1.5 mL tubes. Label with "DNase Stock", the date, and your initials. Store at -20°C.
    - To make the 80 µl of DNase Working Solution required for each sample, add 10 µl of DNase Stock Solution to 70 µl of RDD buffer (provided in the DNase kit). Keep on wet ice or at 4°C until ready to use. Best if made fresh.
- Prepare RNA Carrier - When processing <500 cells, carrier RNA may be added to the lysate before homogenization
    - To make **RNA Carrier Stock Solution**, dissolve the carrier RNA (310 µg) in 1 ml RNase-free water. Store this stock solution at –30°C to –15°C, and use it to make fresh dilutions for each set of RNA preps. The concentration of this stock solution is 310 µg/ml (i.e., 310 ng/µl).
    - To make a **RNA Carrier Working Solution** (4 ng/µl) for 10 preps, add 5 µl stock solution to 34 µl Buffer RLT and mix by pipetting. Add 6 µl of this diluted solution to 54 µl Buffer RLT to give a working solution of 4 ng/µl. Add 5 µl of this solution to the lysate in step 3. Do not add the carrier RNA to the lysate if purifying RNA for use in oligo-dT–based amplification.
- You will need the following additional equipment and reagents:
    - 14.3 M β-mercaptoethanol (β-ME) (commercially available solutions are usually 14.3 M)
    - Microcentrifuge set to room temperature
    - 70% Ethanol and 100% Ethanol
    - Tissue-Lyser

Ensure tissue size is less than 5 mg. Keep tissue frozen until extraction.

1. **Determine the weight of the tissue. As a guide, a 1.5 mm cube (3.4 mm^3) of most animal tissue weighs 3.5–4.5 mg.**

2. **Place the tissues in 2 ml screw cap tubes and add a 5 mm stainless steel bead.**

3. **Add 350 µl of Buffer RLT to each piece of tissue.**
   Note: If processing <2 mg of tissue, 20 ng carrier RNA (5 µl of a 4 ng/µl solution) may be added to the lysate before homogenization.

4. **Disrupt the tissue and homogenize the lysate in the TissueLyser.**
    a. **Place the tubes into the TissuLyser Adapter set, ensuring balance.**
    b. **Homogenize at 30 Hz for 2 min.**
    c. **Flip the orientation of the tubes.**
    d. **Homogenize at 30 Hz for 2 min.**
    e. **Make sure that tissue is homogenized. If not, repeat homogenization.**
    f. **Remove the tubes from the TissueLyser and place at room temperature.**

5. **Centrifuge the lysate for 3 min at maximum speed.**

6. **Add 1 volume of freshly prepared 70% ethanol to the lysate (example: if a lysate has 200 µl, add 200 µl of 70% ethanol), and mix well by pipetting. Do not centrifuge. Proceed immediately to step 7.**

7. **Transfer the sample, including any precipitate, to an RNeasy MinElute spin column placed in a 2 ml collection tube (supplied). Centrifuge for 15 s at ≥8000 x g (≥10,000 rpm). Discard the flow-through.**

8. **Add 350 µl Buffer RW1 to RNeasy column, close lid, centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard flow-through.**

9. **Add DNase I Working Solution (80 µl) directly to RNeasy column membrane, and incubate at room temperature for 15 min.**

10. **Add 350 µl Buffer RW1 to RNeasy MinElute column, close lid, centrifuge for 15 s at ≥8000 x *g*. Discard flow-through.**

11. **Place the RNeasy MinElute spin column in a new 2 ml collection tube (supplied). Add 500 µl Buffer RPE to the spin column. Close the lid, and centrifuge for 15 s at ≥8000 x *g*. Discard the flow-through.**

12. **Add 500 µl of freshly prepared 80% ethanol to the RNeasy MinElute spin column. Close the lid, and centrifuge for 2 min at ≥8000 x *g*. Discard the collection tube.**

13. **Place the RNeasy MinElute spin column in a new 2 ml collection tube (supplied). Open the lid of the spin column, and centrifuge at full speed for 5 min to dry the membrane. Discard the flow-through and collection tube.**

14. **Place the RNeasy MinElute spin column in a new 1.5 ml collection tube (supplied). Add 14 µl pre-warmed RNase-free water directly to the center of the spin column membrane. Close the lid gently, and centrifuge for 1 min at full speed to elute the RNA.**