EMORY UNIVERSITY

Emory Integrated Genomics Core

Emory Integrated Core Facilities

EIGC.002_Appendix I_RNeasy Mini 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

RNeasy Mini kit is used for purification of total RNA from animal cells, animal tissues, bacteria and yeast, and for cleanup of RNA from crude RNA preps and enzymatic reactions (e.g., DNase digestion, proteinase digestion, RNA ligation, and labeling reaction). The main difference between the RNeasy and miRNeasy kits is the option in the latter to enrich for micro RNAs in the sample.

The RNeasy procedure combines the selective binding properties of a silica-based membrane with the speed of micro spin technology. A specialized high-salt buffer system allows up to 100 µg of RNA longer than 200 bases to bind to the RNeasy silica membrane. Biological samples are first lysed and homogenized in the presence of a highly denaturing guanidine-thiocyanate– containing buffer, which immediately inactivates RNAses to ensure purification of intact RNA. Ethanol is added to provide appropriate binding conditions, and the sample is then applied to an RNeasy Mini spin column, where the total RNA binds to the membrane and contaminants are efficiently washed away. High-quality RNA is then eluted in 30–50 µl water.

With the RNeasy 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 RNeasy Kits are designed to purify RNA from small amounts of starting material. They provide a fast and simple method for preparing up to 100 µg total RNA per sample. The purified RNA is ready for use in downstream applications such as: RT-PCR and real-time RT-PCR, Differential display, cDNA synthesis, Northern, dot, and slot blot analyses, Primer extension and Poly A+ RNA selection.

Kit Contents

Rneasy Mini Kit	(50)
Catalog no.	74104
Number of preps	50
RNeasy Mini Spin Columns (pink)	50
Collection Tubes (1.5 ml)	50
Collection Tubes (2 ml)	50
Buffer RTL Plus [a]	45 ml
Buffer RW1 [a]	45 ml
Buffer RPE (concentrate)[b]	11 ml
RNase-Free Water	10 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 Mini Kit (cat. 74104) can be stored at room temperature (15–25°C) for 9 months after receipt 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 tissue 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.
- 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 30 mg. Keep tissue frozen until extraction.

1. **Determine the weight of the tissue and determine the volume of Buffer RLT Plus to prepare.**

Amount of starting material	Volume of Buffer RLT Plus
<20 mg	350 µl or 600 µl*
20–30 mg	600 µl

Note: Ensure that a reducing agent has been added**.**
Note: Use 600 µl Buffer RLT Plus for tissues stabilized in RNAlater®, or for difficult-to-lyse tissues.

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

3. **Add the appropriate volume of Buffer RLT Plus to each piece of tissue.**

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 (example: If lysate has 200 µl, add 200 µl of 70% ethanol) to the lysate and mix well by pipetting. Do not centrifuge. Proceed immediately to step 7.**

7. **Transfer up to 700 µl of the sample, including any precipitate, to an RNeasy 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.**
   Note: Reuse this collection tube through steps 11, 12 and 13.

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 column, close lid, centrifuge for 15 s at ≥8000 x *g*. Discard flow-through.**

11. **Add 700 µl Buffer RW1 to the RNeasy spin column. Close the lid, and centrifuge for 15 s at ≥8000 x g (≥10,000 rpm). Discard the flow-through.**

12. **Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid, and centrifuge for 15 s at ≥8000 x g (≥10,000 rpm). Discard the flow-through.**

13. **Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid, and centrifuge for 2 min at ≥8000 x g (≥10,000 rpm).**

14. **Place the RNeasy spin column in a new 2 ml collection tube (supplied). Discard the old collection tube with the flow-through. Centrifuge at full speed for 1 min to dry the spin column membrane.**

15. **Place the RNeasy spin column in a new 1.5 ml collection tube (supplied). Add 35 µl pre-warmed RNase-free water directly to the spin column membrane.**

16. **Close the lid gently, and centrifuge for 1 min at ≥8000 x g (≥10,000 rpm) to elute the RNA.**

17. **Optional: If the expected RNA yield is >30 µg, repeat step 16 using another 35 µl pre-warmed RNase-free water, or using the eluate from step 15 (if high RNA concentration is required). Reuse the collection tube from step 15.**