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**Emory Integrated  
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**EIGC.002\_Appendix H\_RNeasy Mini Kit for RNA Extraction from  
Cells**

**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 <b>Name</b>	15 June 2020 <b>Date</b>
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Supersedes:	Ashima Amin <b>Name</b>	11 April 2016 <b>Date</b>
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Annual Review and Approval

Michael Zwick, PhD  
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 microspin 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 size distribution of the purified RNA is comparable to that obtained by centrifugation through a CsCl cushion, where small RNAs do not sediment efficiently.

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

<b>Rneasy Mini Kit</b>	<b>(50)</b>
<b>Catalog no.</b>	74104
<b>Number of preps</b>	50
<b>RNeasy Mini Spin Columns (pink)</b>	50
<b>Collection Tubes (1.5 ml)</b>	50
<b>Collection Tubes (2 ml)</b>	50
<b>Buffer RTL Plus <sup>a</sup></b>	45 ml
<b>Buffer RW1 <sup>a</sup></b>	45 ml
<b>Buffer RPE (concentrate)<sup>b</sup></b>	11 ml
<b>RNase-Free Water</b>	10 ml

<sup>a</sup> Contains guanidine salt. Not compatible with disinfecting agents containing bleach; see handbook for safety information.

<sup>b</sup> 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 cell lines rich in RNases, add either 10  $\mu$ l  $\beta$ -mercaptoethanol ( $\beta$ -ME), or 20  $\mu$ l 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  $\mu$ l of water (provided in the DNase kit) to the vial of lyophilized DNase. Mix gently. Remove the stopper and carefully pipet 65  $\mu$ l 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  $\mu$ l of **DNase Working Solution** required for each sample, add 10  $\mu$ l of DNase Stock Solution to 70  $\mu$ l 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  $\beta$ -mercaptoethanol ( $\beta$ -ME) (commercially available solutions are usually 14.3 M)
  - Microcentrifuge set to room temperature
  - 70% Ethanol and 100% Ethanol
  - Blunt-ended needle and syringe

1. **Determine the number of cells in each sample based on the manifest. Each RNeasy Mini Kit column is sufficient to extract RNA from 1-10 million cells.**

**Note:** It is best to start with 3-4 million cells unless you have determined that more cells will not result in DNA contamination.

2. **Add Buffer RLT Plus to each cell pellet:**

Number of pelleted cells	Volume of Buffer RLT Plus
<5 x 10 <sup>6</sup>	350 $\mu$ l
5 x 10 <sup>6</sup> – 1 x 10 <sup>7</sup>	600 $\mu$ l

**Note:** Ensure that a reducing agent has been added if the cell lines are rich in RNases.

3. **Homogenize cells:**
  - a. For <1 x 10<sup>5</sup> cells, vortex sample for 1 minute.
  - b. For 1 x 10<sup>5</sup> – 1 x 10<sup>7</sup> cells, using a 20-gauge needle and syringe, pass the lysate through the needle 5-10 times.
4. **Add freshly prepared 1 volume of 70% ethanol (example: if lysate has 200  $\mu$ l, add 200  $\mu$ l of 70% ethanol) to the lysate and mix well by pipetting. Do not centrifuge. Proceed immediately to step 5.**

5. Transfer up to 700  $\mu$ l of the sample, including any precipitate, to an RNeasy spin column placed in a 2 ml collection tube (supplied). Centrifuge for 15 s at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm). Discard the flow-through.  
  
Note: Reuse this collection tube through steps 9, 10 and 11.
6. Add 350  $\mu$ l Buffer RW1 to RNeasy column, close lid, centrifuge for 15 s at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm). Discard flow-through.
7. Add DNase I Working Solution (80  $\mu$ l) directly to RNeasy column membrane, and incubate at room temperature for 15 min.
8. Add 350  $\mu$ l Buffer RW1 to RNeasy column, close lid, centrifuge for 15 s at  $\geq 8000 \times g$ . Discard flow-through.
9. Add 700  $\mu$ l Buffer RW1 to the RNeasy spin column. Close the lid, and centrifuge for 15 s at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm). Discard the flow-through.
10. Add 500  $\mu$ l Buffer RPE to the RNeasy spin column. Close the lid, and centrifuge for 15 s at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm). Discard the flow-through.
11. Add 500  $\mu$ l Buffer RPE to the RNeasy spin column. Close the lid, and centrifuge for 2 min at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm).
12. 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.
13. Place the RNeasy spin column in a new 1.5 ml collection tube (supplied). Add 35  $\mu$ l pre-warmed RNase-free water directly to the spin column membrane.
14. Close the lid gently, and centrifuge for 1 min at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm) to elute the RNA.
15. Optional: If the expected RNA yield is  $>30 \mu$ g, repeat step 13 using another 35  $\mu$ l of RNase-free water, or using the eluate from step 14 (if high RNA concentration is required). Reuse the collection tube from step 14.