EMORY UNIVERSITY

Emory Integrated Genomics Core

Emory Integrated Core Facilities

EIGC.002_Appendix D_QIAGEN miRNeasy Micro 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 **Name**	15 June 2020 **Date**
Supersedes:	Ashima Amin **Name**	11 April 2016 **Date**

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

The miRNeasy Micro Kit is designed for purification of total RNA, including miRNA and other small RNA molecules, from various sample types, including animal and human cell pellets. The miRNeasy Micro Kit is appropriate for samples with less than 1 million cells.

The miRNeasy Micro Kit combines phenol/guanidine-based lysis of samples and silica- membrane–based purification of total RNA. QIAzol Lysis Reagent is a monophasic solution of phenol and guanidine thiocyanate, designed to facilitate lysis of cells, to inhibit RNases, and to remove most of the cellular DNA and proteins from the lysate by organic extraction.

Cells are suspended in QIAzol Lysis Reagent, supplied with the kit. After addition of chloroform, the homogenate is separated into aqueous and organic phases by centrifugation. RNA partitions to the upper, aqueous phase, while DNA partitions to the interphase and proteins to the lower, organic phase or the interphase.

The upper, aqueous phase is extracted, and ethanol is added to provide appropriate binding conditions for all RNA molecules from 18 nucleotides (nt) upwards. The sample is then applied to the RNeasy Mini spin column, where the total RNA binds to the membrane and phenol and other contaminants are efficiently washed away. High-quality RNA is then eluted in RNase-free water.

Kit Contents

miRNeasyKit	Mini (50)	Micro (50)
Catalog no.	217004	217084
Number of preps	50	50
Spin Columns	50 (RNeasy Mini)	50 (RNeasy MinElute)
Collection Tubes (2 ml)	50	50
Collection Tubes (1.5 ml)	50	50
QIAzol Lysis Reagent [a]	50 ml	50 ml
Buffer RWT [a,b]	15 ml	15 ml
Buffer RPE [c]	11 ml	11 ml
RNase-Free Water	10 ml	10 ml

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

[b] Buffer RWT is supplied as a concentrate. Before using for the first time, add 2 volumes of ethanol (96%–100%) as indicated on the bottle to obtain a working solution

[c] 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.

Procedure

Notes before starting

- Avoid thawing of samples until RNA extraction is to be performed.
- Ensure that Buffer RWT and Buffer RPE have been prepared according to the instructions as indicated on the bottle or the table above.
- 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:
    - QIAzol Lysis Reagent (comes with the kit)
    - Chloroform
    - 80% Ethanol and 100% Ethanol
    - Microcentrifuge set to room temperature
    - Microcentrifuge set to 4°C

All RNA extraction processes should be performed in the chemical hood.

It is important to keep all samples frozen until they are ready to be processed and placed into the QIAzol Lysis Reagent. For this reason, prepare a maximum of 12 samples for extraction per batch.

1. **Determine the number of cells in each sample based on the manifest. Each miRNeasy Micro Kit column is sufficient to extract RNA from up to 1 million cells.**

2. **Add 700 µl QIAzol Lysis Reagent to each sample in a 1.5 ml microcentrifuge tube. Place the tubes on wet ice.**

3. **Include a positive control if there is concern that the sample may be compromised or limiting or if this is the first time that we have performed an extraction for this project.**

   Note: Positive controls can be found in the RNA Control box in the -80°C freezer.

4. **Homogenize cells by vortexing for 1 min.**

5. **Incubate at room temperature for 5 min.**

   Note: This lysate can be stored at -80°C for several months.

6. **Add 0.2 volumes of chloroform to sample (example: 0.2 x 700 µL = 140 µl).**

7. **Shake tubes vigorously for 15 s and incubate at room temperature for 3 min.**

8. **Centrifuge samples at 4°C for 15 min at 12,000 x g.**

9. **Carefully transfer aqueous phase to a new tube, avoiding the organic phase and any white precipitate.**

10. **Measure the aqueous phase with a P1000 and add 1.5 volumes of fresh 100% EtOH. Invert tubes to mix.**

11. **Obtain RNeasy MiniElute spin column (stored at 4°C). Apply 700 µl of sample to the column.**

12. **Centrifuge at 8,000 x g for 15 s at room temperature and discard the flow through.**

13. **Repeat steps 11 and 12 until all of the sample has been applied to the column.**

14. **Add 350 µl Buffer RWT to the column.**

15. **Centrifuge at 8,000 x g for 15 s at room temperature and discard the flow through.**

16. **Add 80 µl DNase Working Solution to the middle of the column. Incubate for 15 min at room temperature.**

17. **Add 350 µl Buffer RWT to the column.**

18. **Centrifuge at 8,000 x g for 15 s at room temperature and discard the flow through.**

19. **Add 500 µl of Buffer RPE to the column.**

20. **Centrifuge at 8,000 x g for 15 s at room temperature and discard the flow through.**

21. **Add 500 µl of 80% Ethanol to the column.**

22. **Centrifuge at 8,000 x g for 15 s at room temperature and discard the flow through.**

23. **Centrifuge the empty column for 5 min at full speed to dry the column.**

24. **Place the RNeasy MiniElute spin column into the labeled tube from step 8.**

25. **Place the column into a new 1.5 mL microcentrifuge tube, and add 14 µl of prewarmed RNase-Free Water to the center of the column.**

26. **Centrifuge at full speed for 1 min at room temperature to elute the RNA.**

27. **Store RNA on wet ice or freeze at -80°C.**