

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 Mini 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

| miRNeasy Mini Kit | Mini (50) | Micro |
|--|------------------|----------------------|
| 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 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.
- Heat RNase-Free Water by placing on top of 70°C heat block.
- 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
 - 70% 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 Mini Kit column is sufficient to extract RNA from 1-10 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.**
6. **Add 0.2 volumes of chloroform to each 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 × 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 a RNeasy Mini spin column. Apply 700 µl of sample to the column.
12. Centrifuge at 8,000 × 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 × 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 × 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 × g for 15 s at room temperature and discard the flow through.
21. Add 500 µl of Buffer RPE to the column.
22. Centrifuge at 8,000 × g for 15 s at room temperature and discard the flow through.
23. Centrifuge the empty column for 1 min at full speed to dry the column.
24. Place the column into a new 1.5 mL microcentrifuge tube and add 35 µl of prewarmed RNase-Free Water to the center of the column.
25. Centrifuge at max speed to elute the RNA.
26. Store RNA on wet ice or freeze at -80°C.