EMORY UNIVERSITY

Emory Integrated Genomics Core

Emory Integrated Core Facilities

EIGC.002_Appendix C_miRNeasy 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**	16 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 added

Introduction

The miRNeasy Mini Kit is designed for purification of total RNA, including miRNA and other small RNA molecules, from various sample types, including tissue. The miRNeasy Mini Kit is appropriate for 5-50 mg of tissue.

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.

Tissue is 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 of tissue 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 tissue 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. Leave each tissue sample on dry ice until ready to thaw in step 6.

1. **Determine the weight of the tissue for each sample. If tissue mass was not provided on the manifest, keeping the samples frozen on dry ice, use a scale to weigh each sample (alternatively, determine the weights of a subset of the samples and estimate the remainder).**

2. **Calculate the volume of QIAzol Lysis Reagent necessary for the sample weight.**

    a. **The amount of QIAzol Lysis Reagent needed to lyse each tissue type is listed below:**

Tissue type	Mass of tissue to use	Volume of QIAzol Lysis Reagent
Most tissues	10-50 mg	700 µl
Liver, thymus, spleen, or any tissue which is stabilized in RNAlater	10-25 mg	700 µl
Adipose tissue	100 mg	700 µl

    b. **If the tissue is more than the recommended weight, then double or triple the volume of QIAzol Lysis Reagent for the sample so that the correct amount of tissue is lysed per column.**

3. **Determine the homogenization method to be used: TissueLyser or Probe Homogenizer. It is recommended that any sample which contains 700 or 1400 µl of QIAzol Lysis Reagent will be processed with the TissueLyser. Any sample which contains 1400 µl or more QIAzol Lysis Reagent can be processed using the Probe Homogenizer.**

4. **For each tissue sample to be processed, add the calculated volume of QIAzol Lysis Reagent to a tube (2 mL screwcap, 5 mL, or 50 mL). For samples to be processed with the TissueLyser, add the QIAzol Lysis Reagent to a 2 mL screwcap tube. For samples to be processed with the Probe Homogenizer, add the QIAzol Lysis Reagent to a 5 mL or 50 mL tube. Place the tubes on wet ice.**

5. **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 of this type.**

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

6. **Transfer the tissue to the tube containing QIAzol Lysis Reagent. Incubate the tubes on ice until all samples have been transferred to QIAzol and homogenized.**

7. **Homogenize the tissue using one of the two following protocols:**
   a. **TissueLyser**
      i. **Add 5 mm stainless steel bead to each sample.**
      ii. **Place the tubes into the TissuLyser Adapter set, ensuring balance.**
      iii. **Homogenize at 30 Hz for 20 s.**
      iv. **Flip the orientation of the tubes.**
      v. **Homogenize at 30 Hz for 20 s.**
      vi. **Remove the tubes from the TissueLyser and place at room temperature.**
   b. **Probe Homogenizer**
      i. **Insert a new probe into the homogenizer.**
      ii. **Insert the probe into the tube containing sample.**
      iii. **Turn on the homogenizer and homogenize until the tissue has dispersed, no more than 30 s.**
      iv. **Immediately place the homogenate onto wet ice until all samples have been homogenized.**
      v. **Change the probe between samples and continue to homogenize samples until all are processed.**

Continue with miRNeasy protocol.

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

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

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

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

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

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

14. **Obtain a RNeasy Mini spin column. Apply 700 µl of sample to the column.**

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

16. **Repeat steps 14 and 15 until all of the sample has been applied to the column.**

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

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

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

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

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

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

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

26. **Centrifuge the empty column for 1 min at full speed to dry the column.**

27. **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.**

28. **Centrifuge at max speed to elute the RNA.**

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