EMORY UNIVERSITY

Emory Integrated Genomics Core

Emory Integrated Core Facilities

EIGC.002_Appendix A_miRNeasy Mini Kit for RNA Extraction from Whole Blood and Buffy Coat

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 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 whole blood and buffy coat.

The miRNeasy Mini Kit combines phenol/guanidine-based lysis of samples and silica- membrane–based purification of total RNA. Trizol LS 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.

Blood is suspended in Trizol LS. 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 stored blood 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:
    - Trizol LS
    - 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 blood samples frozen until they are ready to be processed and placed into the Trizol LS. For this reason, prepare a maximum of 12 samples for extraction per batch. Leave each blood sample on dry ice until ready to thaw in step 2.

1. **For each blood or buffy coat sample to be processed, add 600 µl of Trizol LS to 1.5 mL tubes. Additionally, 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.

2. **Thaw up to 4 samples in 37°C water bath for 1 min or until just thawed (temperatures above 4°C can result in degraded RNA).**

3. **Gently mix sample by inverting the tube 3 times, spin down briefly to collect, and place on wet ice.**

4. **Gently pipette mix the sample and transfer 200 µl directly into the 600 µl Trizol LS using a P1000 pipette. Place any remaining sample back onto dry ice.**

   Note: You can refreeze these samples at -80°C at the end of the extraction, and these samples can be used for DNA extractions. It is not recommended to extract RNA from re-frozen samples.

5. **Immediately shake the 800 µl sample for 30 s by hand, and then place on wet ice until all samples have been processed.**

6. **Continue with remaining samples until all of the blood samples have been added to Trizol LS, repeating steps 2-5.**

Continue with miRNeasy protocol.

7. **Incubate blood-Trizol samples at room temperature for 5 min.**

8. **Add 0.2 volumes of chloroform to each sample (example: 0.2 x 800 µl = 160 µl).**

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

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

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

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

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

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

15. **Repeat steps 13 and 14 until all of the sample has been applied to the column.**

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

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

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

19. **Add 350 µl Buffer RWT 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 Buffer RPE to the column.**

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

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

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

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

26. **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.**

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

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