FISHER MOLECULAR BIOLOGY

miRNA Isolation Kit RE-032 & RE-033

miRNA Isolation Kit is designed for purification of microRNAs (miRNAs) and other small cellular RNAs from tissue samples or cultured cells. Purification of miRNAs allows research into biological significant pathways for regulation of gene regulation.

The standard protocols for isolating total RNA and mRNA are not optimized for isolation of small RNA molecules and result in the loss of substantial amounts of miRNAs and other small RNA. In addition, removal of the predominant larger RNAs is required for accurate analysis of miRNA expression by qPCR or microarray analysis.

This kit is specifically designed for purification of small RNA with minimal contamination from large RNA molecules or genomic DNA. The method employs a spin column with a silica-based fiber matrix that binds RNA in the presence of a chaotropic salt. The method is based on the selective binding of RNA molecules of different sizes to the silica-based fiber matrix when different ethanol concentrations are present in the solvent.

Kit Contents:

Cat. No:	**RE-032S (5 Rxn)**	**RE-033 (100 Rxn)**	**RE-032 (50 Rxn)**
Lysis Buffer	1.5 ml	25 ml	12 ml
2M NaOAc, pH 5.2	150 µl	2.5 ml	1.2 ml
Wash Buffer (concentrated)[a]	0.3 ml	5 ml	3 ml
Release Buffer	0.32 ml	5.5 ml	2.8 ml
RNA Column	10 pcs	200 pcs	100 pcs
Collection Tube	10 pcs	200 pcs	100 pcs

Preparation of Wash Buffer by adding ethanol (96 ~ 100%) and **Store at RT.**			
Ethanol volume for Wash Buffer **a**	1.2 ml	20 ml	12 ml

Specification:

Principle:	mini spin column (silica matrix)
Sample size:	up to 1 x10^6 Cultured cells up to 100 mg tissue
Operation time:	~ 30 minutes
Column applicability:	centrifugation and vaccum

Additional requirement to be provided by user

1. Microcentrifuge capable of speed at ~12,000 rpm
2. 1.5 ml microcentrifuge tube
3. 96~100 % ethanol
4. Chlorofrom
5. Vortex
6. Water bath or dry bath

Important Notes:

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. Store the kit at room temperaturel.
3. Caution: phenol and chlorofrom are hazardous to human health. perform the procedures involving phenol and chlorofrom in a chemical fume hood.
4. Add required volume of ethanol (96- 100 %) to Wash Buffer when first open. **Store the solution at room** temperature.

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36 Terry Drive
Trevose, PA 19048 - USA

Genernal Protocol:

Read the Important Note before starting the following steps. HINT:

Preheat Release Buffer to 65°C for step 15.

1. Add 200 µl Lysis Buffer into the tube containing up to 100 mg tissue or $1x10^6$ cultured cell pellet.
2. Vigorous mixing by vortexing. Incubate at room temperature for 10 minutes.
3. Add 20 µl 2M NaOAc, pH 5.2.
4. Add 180 µl ddH2O saturated phenol and 40 µl chloroform into the tube, vortex vigorously for 2 minutes.
5. Centrifuge at 12,000 rpm for 3 minutes. Transfer the upper phase into a clean tube.
6. Add ethanol to 35% volume (ex., add 108 µl ethanol to 200 µl upper phase). Mix well.
7. Transfer to the RNA Column in the Collection Tube. Incubate for 1 minute.
8. Centrifuge at 12,000 rpm for 30 seconds. Collect the filtrate.
9. Add ethanol to 70% volume (ex., add 338 µl ethanol to 290 µl upper phase). Mix well.
10. Transfer to another RNA Column in the Collection Tube. Incubate for 1 minute.
11. Centrifuge at 12,000 rpm for 30 seconds (miRNA bound to the column membrane).
12. Add 200 µl Wash Buffer (ethanol added). Incubate for 1 minute.
13. Centrifuge at 12,000 rpm for 1 minute to completely remove the residue liquid.
14. Put the RNA Column to a clean 1.5 ml tube.
15. Add 50 µl Release Buffer (preheated to 65°C) to the center of column. Incubate for 3 minutes.
16. Centrifuge at 12,000 rpm for 3 minute to recover miRNA. (Note: The purified miRNA can be further concentrated by a standard ethanol precipitation procedure and then re-dissolved in a small volume ddH2O or TE, pH 8.0).
17. Use 1/5 volume to run on a mini agarose gel (or more accurately, a polyacrylamide gel) to check its quality. The majority of RNA visible on the gel should be <100 nt in size, with the major bands corresponding to tRNAs. The 5S and 5.8S rRNA species may also be visible. These tRNA and small rRNA bands should be clear and distinct. miRNA (21-22 nt) are typically not visible on the gel image.

Special Protocol for isolation of miRNA from serum

Read the Important Note before starting the following steps. HINT: Preheat Release Buffer to 65°C for step 15.

1. Add 2.5 volumes of Lysis Buffer into the tube containing 1 volume of serum sample.

•For example: add 500 Ul of Lysis Buffer to 200 Ul of serum sample.

•If the volume of serum sample is more than 200 Ul, separate it into multiple tubes.

2. Vigorous mixing by vortexing. Incubate at room temperature for 10 minutes.

3. Add 0.25 volume of 2M NaOAc, pH 5.2 to the sample mixture.

•For example: add 50 Ul of 2M NaOAc, pH 5.2 to sample mixture as the starting sample is 200 Ul of serum.

4. Add 2.25 volumes of ddH2O saturated phenol and 1 volume of chloroform into the tube, vortex vigorously for 2 minutes.

•For example: add 450 Ul of ddH2O saturated phenol and 200 µl of chloroform to sample mixture as the starting sample is 200 Ul of serum.

5. Centrifuge at 12,000 rpm for 3 minutes. Transfer the upper phase into a clean tube.

6. Add ethanol to 35% volume (ex., add 108 µl ethanol to 200 µl upper phase). Mix well.

7. Transfer to the RNA Column in the Collection Tube. Incubate for 1 minute.

8. Centrifuge at 12,000 rpm for 30 seconds. Collect the filtrate.

9. Add ethanol to 70% volume (ex., add 338 µl ethanol to 290 µl upper phase). Mix well.

10. Transfer to another RNA Column in the Collection Tube. Incubate for 1 minute.

11. Centrifuge at 12,000 rpm for 30 seconds (miRNA bound to the column membrane).

12. Add 200 µl Wash Buffer (ethanol added). Incubate for 1 minute.

13. Centrifuge at 12,000 rpm for 1 minute to completely remove the residue liquid.

14. Put the RNA Column to a clean 1.5 ml tube.

15. Add 40 ~ 50 µl Release Buffer (preheated to 65°C) to the center of column. Incubate for 3 minutes.

16. Centrifuge at 12,000 rpm for 3 minute to recover miRNA. (Note: The purified miRNA can be further concentrated by a standard ethanol precipitation procedure and then re-dissolved in a small volume ddH2O or TE, pH 8.0).

17. Use 1/5 volume to run on a mini agarose gel (or more accurately, a polyacrylamide gel) to check its quality. The majority of RNA visible on the gel should be <100 nt in size, with the major bands

corresponding to tRNAs. The 5S and 5.8S rRNA species may also be visible. These tRNA and small rRNA bands should be clear and distinct. miRNA (21-22 nt) are typically not visible on the gel image.