User Manual

FavorPrep™ miRNA Extractiont Mini Kit

Cat.: FAMI1020
FAMI1023
FAMI1024
(For Research Use Only)

Introduction:

FavorPrep™ miRNA Exrtraction Mini 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 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:	**FAMI1020 (4 preps)**	**FAMI1023 (50 preps)**	**FAMI1024 (100 preps)**
Lysis Buffer	1.5 ml	12 ml	25 ml
2 M NaOAc, pH 5.2	150 µl	1.2 ml	2.5 ml
Wash Buffer 1	3 ml	30 ml	60 ml
Wash Buffer 2 (Concentrate)[a]	1.5 ml	15 ml	35 ml
RNase-Free Water	0.5 ml	6 ml	6 ml
RNA Columns	8 pcs	100 pcs	200 pcs
Collection Tubes	8 pcs	100 pcs	200 pcs
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Preparation of Wash Buffer 2 by adding ethanol (96~100%) and **Store at RT.**			
Ethanol volume for Wash Buffer 2 [a]	6 ml	60 ml	140 ml

Specification:

Principle:	mini spin column (silica matrix)
Sample size:	up to 1×10^6 cultured cells up to 50 mg tissue
Operation time:	30 minutes
Column applicability:	centrifugation and vacuum

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. Water-saturated phenol
5. Chloroform
6. Vortex
7. 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 temperature.
3. Caution: phenol and chloroform 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 2 when first open. **Store the solution at room temperature.**

Protocol:

Read the Important Note before starting the following steps.

HINT: Preheat RNase-Free Water to 65°C for step 16.

1. Add 200 µl Lysis Buffer into the tube containing up to 50 mg tissue or 1×10^6 cultured cell pellet.

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

3. Add 20 µl 2 M NaOAc, pH 5.2.

4. Add 180 µl water-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 carefully into a new 1.5 ml centrifuge tube.
   IMPORTANT! Avoid picking up any of the interphase or organic layer when transferring the aqueous phase (upper phase).

6. Add absolute ethanol (0.54× volume of upper phase) to the upper phase and mix well by shaking vigorously.
   -If the upper phase volume is 200 µl, add 108 µl of absolute ethanol to upper phase. The final ethanol concentration of whole mixture will be 35%.

7. Place a RNA Column in a Collection Tube and transfer the ethanol-added mixture to the RNA Column. Incubate for 1 minute.

8. Centrifuge at 12,000 rpm for 30 seconds (Large RNA bound to the column membrane).

9. Transfer the filtrate to a new 1.5 ml microcentrifuge tube. Add absolute ethanol (1.1× volume of filtrate) then mix well by shaking vigorously.
   -If the filtrate volume is 308 µl, add 338 µl of absolute ethanol. The final ethanol concentration of whole mixture will be 70%.

10. Place a new RNA Column in a Collection Tube then transfer the mixture to the RNA Column. Incubate for 1 minute.

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

12. Add 500 µl of Wash Buffer 1 to the RNA Column. Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through and return the RNA column to the collection tube.

13. Add 700 µl of Wash Buffer 2 (ethanol added) to the RNA Column. Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through and return the RNA Column to the collection tube.

14. Add 400 µl of Wash Buffer 2 (ethanol added) to the RNA column. Centrifuge at 12,000 rpm for 2 minutes to dry the column. Discard the flow-through and the collection tube.

15. Put the RNA Column to a new 1.5 ml centrifuge tube (not provided).

16. Add 30~50 µl RNase-Free Water (preheated to 65°C) to the center of column. Incubate for 3 minutes.

17. 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 ddH_2O or TE, pH 8.0).

18. 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.