



FavorPrep™ miRNA Extraction HE Mini Kit

■ Kit Contents

Cat. No.	FAMI0030 (4 Preps)	FAMI0033 (50 Preps)	FAMI0034 (100 Preps)
FavorPrep™ Tri-RNA Reagent	4.5 ml	55 ml	110 ml
Wash Buffer 1	3 ml	40 ml	80 ml
Wash Buffer 2 (Concentrate) ▲	1.5 ml	20 ml	40 ml
RNase-Free Water	0.5 ml	6 ml	6 ml
HE Columns	4 pcs x 2	50 pcs x 2	50 pcs x 4
HE Collection Tubes	8 pcs	100 pcs	100 pcs x 2
Elution Tubes	4 pcs	50 pcs	100 pcs
User Manual	1	1	1
Preparation of Wash Buffer 2 by adding 96~100% ethanol.			
Volume of Ethanol for Wash Buffer 2 ▲	6 ml	80 ml	160 ml

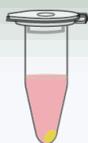
1. All kit components are shipped at room temperature and should be stored at room temperatures between 15~25°C upon receipt, **except FavorPrep™ Tri-RNA Reagent.**
2. Store FavorPrep™ Tri-RNA Reagent at 4°C upon receipt.

■ Specification

Format/Principle	Spin Column (silica matrix)
Binding Capacity	≤250 µg RNA/Column
Operation Time	<40 mins
Sample Size	≤30 mg Tissue ≤1~5 × 10 ⁶ Cultured Cell ≤ 200 µl exosomes
RNA Yield	Total RNA: ≤40 µg Small RNA: ≤7 µg
Elution Volume	30 µl

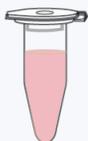
■ Procedure Overview: Purification of miRNA-Enriched Fractions

Tissue or Culture Cell
Sample



- Lysis the sample in 1 ml FavorPrep™ Tri-RNA Reagent.
- Stand the sample for 5 mins at room temperature.

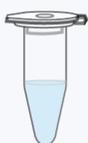
↻ Centrifuge
18,000 xg, 3 mins



- Add 200 µl chloroform.
- Vigorous vortexing for 2 mins.

HE Column

↻ Centrifuge
18,000 xg, 1 min



- Transfer upper phase.
- Add 0.54x ethanol (96~100%).



- Transfer mixture to HE Column.
- Binding large RNA.
- Save the filtrate.



- Transfer the filtrate to new tube.
- Add 1.1x ethanol (96~100%).

↻ Centrifuge
18,000 xg, 1 min



- Transfer mixture to HE Column.
- Binding small RNA.

↻ Centrifuge
18,000 xg, 1 min



- Add 700 µl Wash Buffer 1 (ethanol contained) and centrifuge
- Add 750 µl Wash Buffer 2 (ethanol contained) and dry the column membrane.

↻ Centrifuge
18,000 xg, 3 mins



- Elution (add 30 µl RNase-Free Water).
- Stand the column for 5 mins at room temperature.
- Obtain purified RNA.

■ Preparation Before Starting

1. Add the indicated volume of ethanol (96~100%) into the **Wash Buffer 2**, mix well, and store at room temperature.
2. Additional materials: DNase I (Optional), RNase-free 96~100% ethanol, RNase-free 70% ethanol, 1.5 ml and 2.0 ml microcentrifuge tube.
3. Make sure all materials are RNase-free when handling RNA.
4. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
5. **Caution:** FavorPrep™ Tri-RNA Reagent is hazardous to human health. Perform the procedures involving Tri-RNA Reagent in a chemical fume hood. Tri-RNA Reagent should be sealed tightly, avoid light and store at 4°C after use.
6. (Optional) For long-term RNA storage, immerse the tissue or cell in FavorPrep™ NApreserve Reagent (Cat. No. FNPR1084) as instructed in the user manual.
7. (Optional) Prepare DNase I working solution following the user guide of FavorPrep™ DNase I Solution (Cat. No. FADI209) and make the final concentration of DNase I to 0.25 U/μl.
8. All centrifugation steps should be performed at **18,000 xg**.

■ Protocol for Purification of Total RNA, Including miRNA

1. Sample preparation
 - a. Cells: Collect 1~5 × 10⁶ cells by centrifuging at 300 xg for 5 mins. Remove all the supernatant.
 - b. Tissues: Weight up to 30 mg of tissue sample to a microcentrifuge tube (not provided).
2. Add 1 ml of **FavorPrep™ Tri-RNA Reagent** to lyse sample.
 - a. Cells: Repetitive pipetting or vortex vigorously to lyse cell.
 - b. Tissues: Homogenize the tissue by using a rotor-stator homogenizer or by passing the sample lysate through a 20-G needle syringe 10 times.
3. Stand the homogenates for 5 mins at room temperature.
4. Add 200 μl chloroform into the tube, vortex vigorously for 2 mins.
5. Centrifuge for 3 mins. 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 RNase-free 96~100% ethanol (1.5x volume of upper phase) to the upper phase and mix thoroughly.
 - If the upper phase volume is 500 μl, add 750 μl of RNase-free 96~100% ethanol to upper phase.
7. Place a **HE Column** to a **HE Collection Tube** and transfer 900 μl of the sample mixture to the HE Column. Centrifuge for 1 min, discard the flow-through and return the HE Column back to the HE Collection Tube.

8. Repeat step 7 for remaining sample mixture.
9. **(Optional step)** On-column DNase I digestion, if eliminating gDNA contamination in RNA sample is required, proceed the “**On-column DNase I digestion**”.
10. Add 700 μ l of **Wash Buffer 1** to the HE Column, centrifuge for 1 min. Discard the flow-through and place HE Column back to the HE Collection Tube.
11. Add 750 μ l of **Wash Buffer 2** (ethanol contained) to the HE Column, centrifuge for 1 min. Discard the flow-through and place the HE Column back to the HE Collection Tube.
12. Add 750 μ l Wash Buffer 2 (ethanol contained) to the HE Column. Centrifuge for 2 mins to dry the membrane directly. Discard flow-through and HE Collection Tube.
13. Place the HE Column to an **Elution Tube**, then add 30 μ l of **RNase-Free Water** to the membrane center of the HE Column. Stand the HE Column for 5 mins.
 - **IMPORTANT!** For effective elution, make sure that RNase-Free Water is dispensed onto the membrane center and absorbed completely.
15. Centrifuge the HE Column for 3 mins to elute RNA.
16. Store RNA at -70°C .

■ Protocol for Purification of miRNA-Enriched Fractions from Tissue or Cultured Cells

1. Sample preparation
 - a. Cells: Collect $1\sim 5 \times 10^6$ cells by centrifuging at 300 xg for 5 mins. Remove all the supernatant.
 - b. Tissues: Weight up to 30 mg of tissue sample to a microcentrifuge tube (not provided).
2. Add 1 ml of **FavorPrep™ Tri-RNA Reagent** to lyse sample.
 - a. Cells: Repetitive pipetting or vortex vigorously to lyse cell.
 - b. Tissues: Homogenize the tissue by using a rotor-stator homogenizer or by passing the sample lysate through a 20-G needle syringe 10 times.
3. Stand the homogenates for 5 mins at room temperature.
4. Add 200 μ l chloroform into the tube, vortex vigorously for 2 mins.
5. Centrifuge for 3 mins. 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 RNase-free 96~100% ethanol (0.54x volume of upper phase) to the upper phase and mix thoroughly.
 - If the upper phase volume is 500 μ l, add 270 μ l of RNase-free 96~100% ethanol to upper phase.
7. Place a **HE Column** to a **HE Collection Tube** and transfer the sample mixture to the HE Column. Centrifuge for 1 min, save the filtrate (Large RNA bound to the column membrane).

8. Transfer the filtrate to a new 2.0 ml microcentrifuge tube. Add RNase-free 96~100% ethanol (1.1x volume of filtrate) then mix well by shaking vigorously.
 - If the filtrate volume is 770 μ l, add 847 μ l of RNase-free 96~100% ethanol.
9. Place a new HE Column in a HE Collection Tube then transfer 900 μ l of the ethanol-added filtrate to the HE Column. Centrifuge for 1 min, discard the flow-through and return the HE Column back to the HE Collection Tube (Small RNA bound to the column membrane).
10. Repeat step 9 for remaining sample mixture.
11. Add 700 μ l of Wash Buffer 1 to the HE Column, centrifuge for 1 min. Discard the flow-through and place HE Column back to the HE Collection Tube.
12. Add 750 μ l of **Wash Buffer 2** (ethanol contained) to the HE Column, centrifuge for 1 min. Discard the flow-through and place the HE Column back to the HE Collection Tube.
12. Add 750 μ l Wash Buffer 2 (ethanol contained) to the HE Column. Centrifuge for 2 mins to dry the membrane directly. Discard flow-through and HE Collection Tube.
13. Place the HE Column to an **Elution Tube**, then add 30 μ l of **RNase-Free Water** to the membrane center of the HE Column. Stand the HE Column for 5 mins.
 - **IMPORTANT!** For effective elution, make sure that RNase-Free Water is dispensed onto the membrane center and absorbed completely.
15. Centrifuge the HE Column for 3 mins to elute RNA.
16. Store RNA at -70°C.

■ Protocol for Purification of miRNA form Exosome

1. Add 1 ml **FavorPrep™ Tri-RNA Reagent** into the tube containing up to 200 μ l exosome sample.
 - **IMPORTANT!** If the initial sample volume exceeds 200 μ l, the samples will need to be processed in multiple loads due to limitations of buffer capacity.
2. Vigorous mixing by vortexing thoroughly. Stand the homogenates for 5 mins at room temperature.
3. Add 200 μ l chloroform into the tube, vortex vigorously for 2 mins.
4. Centrifuge for 3 mins. 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 RNase-free 96~100% ethanol (2.35x volume of upper phase) to the upper phase and mix thoroughly.
 - If the upper phase volume is 500 μ l, add 1175 μ l of RNase-free 96~100% ethanol to upper phase.
7. Follow the steps from Step 9 of "**Protocol for Purification of miRNA-Enriched Fractions from Tissue or Cultured Cell**".

■ Protocol for On-column DNase I digestion

Follow the steps from Step 9 of “Protocol for Purification of Total RNA, Including miRNA” to eliminate genomic DNA contamination

1. Add 350 μ l of **Wash Buffer 1** to the HE Column. Centrifuge for 1 min. Discard the flow-through and return the HE Column back to the HE Collection Tube.
2. Add 900 μ l of **RNase-free 70% ethanol** to the HE Column. Centrifuge for 1min. Discard the flow-through and return the HE Column back to the HE Collection Tube.
3. Add 40 μ l of **RNase-free DNase I solution** (0.25 U/ μ l, not provided) to the membrane center of the HE Column.
4. Place the column on the benchtop for 15 mins.
5. Add 350 μ l of **Wash Buffer 1** to the HE Column. Centrifuge for 1 min. Discard the flow-through and return the HE Column back to the HE Collection Tube.
6. After DNase I treatment, proceed step 11. of “Protocol for Purification of Total RNA, Including miRNA”.

For more product information, please visit <https://www.favorgen.com/>
For technical assistance, please email us at Technical@favorgen.com

This product is for research use only. It is not intended to be used for therapeutic or diagnostic purposes.