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 x 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.
		• Transfer upper phase. • Add 0.54x ethanol (96~100%).
HE Column Centrifuge 18,000 xg, 1 min		• 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 x 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 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 µl 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 µl 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 µl 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 µl 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 1~5 x 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 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 (0.54x volume of upper phase) to the upper phase and mix thoroughly.
    - If the upper phase volume is 500 µl, add 270 µl 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 µl, add 847 µl of RNase-free 96~100% ethanol.
9. Place a new HE Column in a HE Collection Tube then transfer 900 µl 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 µl 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 µl 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 µl 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 µl 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 µl exosome sample.
   - **IMPORTANT!** If the initial sample volume exceeds 200 µl, 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 µl 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 µl, add 1175 µl 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 µl 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 µl 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 µl of **RNase-free DNase I solution** (0.25 U/µl, not provided) to the membrane center of the HE Column.
4. Place the column on the benchtop for 15 mins.
5. Add 350 µl 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".