FavorPrep™ Tissue Total RNA Extraction HE Mini Kit

Kit Contents

Cat. No.	FATR0030 (4 Preps)	FATR0033 (50 Preps)	FATR0034 (100 Preps)
FARB Buffer	1.5 ml x 2	30 ml	60 ml
Wash Buffer 1	1.5 ml x 2	30 ml	60 ml
Wash Buffer 2 (Concentrate)▲	1.5 ml	20 ml	35 ml
RNase-Free Water	0.5 ml	6 ml	8 ml
Filter Columns	4 pcs	50 pcs	50 pcs x 2
HE Columns	4 pcs	50 pcs	50 pcs x 2
HE Collection Tubes	4 pcs x 3	50 pcs x 3	100 pcs x 3
Elution Tubes	4 pcs	50 pcs	100 pcs
Micropestles	4 pcs	50 pcs	50 pcs x 2
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	140 ml

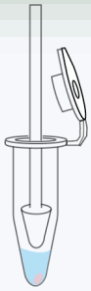
All kit components are shipped at room temperature and should be stored at room temperatures between 15~25°C upon receipt.

Specification

Format/Principle	Spin column (Silica matrix)
Binding Capacity	≤250 µg RNA/Column
Operation Time	<45 mins
Sample Size	Tissue: ≤ 30 mg Cultured cells: 10^1~10^7 cells
RNA Yield	≤75 µg
Elution Volume	30 µl

Procedure Overview

Animal tissues

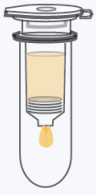


- Cut the tissue (up to 30 mg) into a 1.5 ml tube (Not provided).
- Add 550 µl β-ME-FARB mixture.
- Homogenize using a homogenizer or Micropestle.
- Stand at room temperature for 5 mins.

Animal cells

Centrifuge
18,000 xg, 5 mins

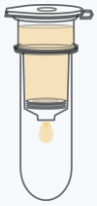
- Centrifuge to pellet the cells up to 1 x 10^7.
- Discard the supernatant.
- Add 550 µl β-ME-FARB mixture.
- Vortex for 1 min.



Centrifuge
18,000 xg, 3 mins

- Transfer entire mixture into Filter Column.
- Transfer all the supernatant of the filtrate into a new 1.5 ml tube.

HE Column



Centrifuge
18,000 xg, 3 mins

- Add 1x volume of RNase-Free 70% ethanol.
- Transfer up to 1000 µl of the sample mixture into the HE Column for RNA binding. (Repeat the step if the mixture volume exceeds 1000 µl.)

Centrifuge
18,000 xg, 1 min

Centrifuge
18,000 xg, 2 mins

- Add 500 µl Wash Buffer 1 and centrifuge.
- Add 750 µl Wash Buffer 2 (ethanol contained) and centrifuge
- Add 750 µl Wash Buffer 2 (ethanol contained) and dry the column membrane.

Centrifuge
18,000 xg, 2 mins

- Add 30 µl RNase-Free Water.
- Stand the column for 5 mins at room temperature.
- Obtain purified RNA.

■ Preparation Before Starting

1. Additional materials: β-mercaptoethanol (β-ME), RNase-Free 70% and 96~100% ethanol, 20-G needle with syringe (optional), and DNase I (optional).
2. (Optional) For long-term RNA storage, immerse the animal tissue in FavorPrep™ NApreserve Reagent (Cat. No. FNPR1084) as instructed in the user manual.
3. (Optional) Prepare DNase I working solution following the user guide of FavorPrep™ DNase I Solution (Cat. No. FADI209) and make the final concentration of 0.25 U/μl.
4. For a fresh preparation of **β-ME-FARB mixture**, premix 5.5 μl of β-ME and 550 μl of **FABR Buffer** per sample before executing RNA extraction.
5. **Caution: β-ME is hazardous to human health. Always perform procedures involving β-ME in a fume hood.**
6. Add the indicated volume of ethanol (96~100%) into **Wash Buffer 2**, mix well, and store at room temperature.

■ Sample pre-processing

For Animal Tissues

1. Add 550 μl of **β-ME-FARB mixture** to the tissue samples up to 30 mg.
   - **Note:** For RNA-rich or soft organ tissues (e.g., liver or kidney), limit the sample input to ≤10~15 mg to avoid column overload.
2. Homogenize the sample using a homogenizer or **Micropestle**.
   - **Note:** If debris remains, pass the lysate through a 20-G needle syringe 10 times.
3. Incubate the sample at room temperature for 5 mins.
   - **Note:** If the lysate appears gel-like or stringy, reduce the input amount or split across multiple tubes.
4. Proceed to the **General Protocol**.

For Cultured Cells

1. Transfer up to 1 x 10^7 cultured cells to a 1.5 ml microcentrifuge tube (not provided).
2. Add 550 μl of **β-ME-FARB mixture** and lyse the cells by vortexing vigorously for 1 min.
3. Proceed to the **General Protocol**.

■ General Protocol

- **Note:** All centrifugation steps should be performed at **18,000 xg** at room temperature.

1. Ensure the sample has been appropriately processed as instructed in the **Sample Pre-processing** section.

2. Place a **Filter Column** in an **HE Collection Tube** and transfer the entire mixture to the Filter Column.
3. Centrifuge for 3 mins. Carefully transfer the supernatant of filtrate into a new a 1.5 ml microcentrifuge tube (not provided).
   - **Note:** Avoid pipetting any debris and pellets when transferring the supernatant.
4. Measure the volume of the supernatant and add 1x volumes of RNase-Free 70% ethanol to the sample mixture. Mix thoroughly by vortexing.
5. Place an **HE Column** in an **HE Collection Tube**. Transfer all the mixture (including any precipitate) into the HE Column. Centrifuge for 3 mins and discard the flow-through. Repeat this step until all mixture has been processed.
6. **(Optional) DNase I digestion.** To eliminate genomic DNA contamination, follow the steps from a.
   a. Add 250 µl of **Wash Buffer 1** to the HE Column, and centrifuge for 1 min. Discard the flow-through and place the HE Column in the HE Collection Tube.
   b. Add 750 µl of **RNase-Free 70% ethanol** to the HE Column, and centrifuge at 18,000 xg for 1 min. Discard the flow-through and place the HE Column in the HE Collection Tube.
   c. Add 60 µl of **RNase-Free DNase I solution** (0.25 U/µl, not provided) to the membrane center of the HE Column. Place the column on the benchtop for 15 mins.
   d. Add 250 µl of **Wash Buffer 1** to the HE Column , and centrifuge for 1 min. Discard the flow-through and place the HE Column in the HE Collection Tube.
   e. Proceed to step 8.
7. Add 500 µl **Wash Buffer 1** to the HE Column. Centrifuge for 1 min then discard the flow-through.
8. Add 750 µl **Wash Buffer 2** (ethanol contained) to the HE Column. Centrifuge for 1 min then discard flow-through.
9. 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.
   - **Important step!** Don't let the tip of the column touch the flow-through. If in doubt, spin again to remove all residual ethanol.
10. Place the HE Column in an **Elution Tube**, then add 30 µl **RNase-Free Water** directly onto the membrane. Stand the HE Column for 5 mins.
    - **Important step!** For effective elution, ensure that the elution solution is dispensed onto the membrane center and absorbed completely.
11. Centrifuge for 2 mins to elute the RNA.