User Manual

FavorPrep™ 96-Well Total RNA Kit

(For Research Use Only)

- For 96-well high-throughput extraction of total RNA from aminal cells or tissues

Kit Contents:

Cat. No.: (Q'ty)	FATRE 96001 (1 plate)	FATRE 96002 (2 plates)	FATRE 96004 (4 plates)
Lysis Buffer	60 ml	120 ml	120 ml x 2
Wash Buffer 1 * (concentrate)	55 ml	110 ml	110 ml x 2
Wash Buffer 2 ■ (concentrate)	25 ml	50 ml	50 ml x 2
RNase-Free Water	15 ml	30 ml	30 ml x 2
Filter Plate (96-Well RNA plate)	1 plate	2 plates	4 plates
Collection Plate (96-Well 2 ml Plate)	4 plates	8 plates	16 plates
Elution Plate (96-Well PCR plate)	1 plate	2 plates	4 plates
Adhesive Film	2 pcs	4 pcs	8 plates

Preparation of working buffers

Add RNase-free ethanol (96~100%) to Wash Buffer 1 and Wash Buffer 2 when first use.

	FATRE 96001	FATRE 96002	FATRE 96004
* Ethanol volume for Wash Buffer 1	10 ml	20 ml	
■ Ethanol volume for Wash Buffer 2	100 ml	200 ml	

Related products can be ordered from Favorgen

	Cat. No:	Content:	Description:
DNase I Reaction Set (50 reactions)	FADASE-50	1. DNase I (lyophilized), 5 tubes (10 reactions/ tube) 2. RNase-free water 3. DNase I Reaction Buffer	1. Molecular Biology Grade. 2. Chromatographically purified to remove RNase and protease. 3. Store at 2~8 °C.

Quality Control

The quality of 96-Well Total RNA Kit is tested on a lot-to-lot basis. The purified RNA is checked by real-time PCR and capillary electrophoresis,

Specification:

Principle: Filter Plate (silica membrane)
Sample size: up to 1 x 10^7 animal cells or 50 mg tissues / preparation
Processing: centrifugation protocol or vacuum & centrifugation protocol
Operation time: < within 1 hr/ 96 preparation
RNA Binding capacity: up to 75 µg/ well
Elution volume: 50 ~ 75 µl

Reagent to be provided by user

1. ß-mercaptoethanol (ß-Me)
2. 96 ~100 % RNase - free ethanol
3. 70 % RNase - free ethanol

Important notes:

1. Make sure everything is RNase-free when handling this kit.
2. Buffers provided in this system contain irritants. Wear gloves and lab coat when andling these buffers.
3. **Caution: ß-mercaptoethanol is hazardous to human health. perform the procedures involving ß-M in a chemical fume hood.**
4. Add RNase-free ethanol (96~100%) to Wash Buffer 1 and Wash Buffer 2 when first use.
5. Prepare RNase-free DNase 1 reaction buffer (1M NaCl, 10 mM MnCl2, 20 mM Tris-HCl, pH 7.0 at 25°C) and make the final concentration of DNase I to 0.5 U/ µl.
6. Equipments required:
   - For centrifugation protocol: A centrifuge is required, capable of 5,600 ~ 6,000 X g, with a swing -bucket rotor and the adaptor for 96-well plates.
   - For vacuum protocol: A vacuun manifold for 96-well plate and a vaccum source reached to 15 inches Hg are required.

   (Alternative): If using centrifugation for Elution Step (STEP 9), a centrifuge equiment is required, capable of 5,600 ~ 6,000 X g, with a swing -bucket rotor and the adaptor for 96-well plate.

Sample amount and yield

Sample	Recommended amount of sample used	Average yield (µg)
Animal cells (up to 1 x 10^7)	HeLa, 1 x 10^6 cells	15
High yield Tissue (Mouse)(up to 20 mg)	Liver, 10 mg	30~50
	Spleen, 10 mg	40~60
Low yield Tissue (Mouse)(up to 50 mg)	Embryo, 10 mg	10~25
	Heart, 10 mg	5~15
	Brain, 10 mg	5~15
	Kidney, 10 mg	20~30
	Lung, 10 mg	10~20
	Intestine, 10 mg	10~30

Brief procedure:

• STEP 1. Sample preparation and lysis

• Collect samples in a Collection Plate (first collection plate) → • Add Lysis Buffer → • Disrupt the samples → • Stand at room temperature for 5 min

• STEP 2. Clarify lysate

• Seal with Adhesive Film.
• Centrifuge at 5,600 – 6,000 x g for 10 min

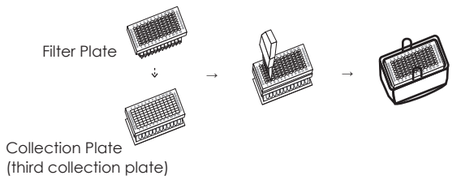
• STEP 3. Adjust binding condition:

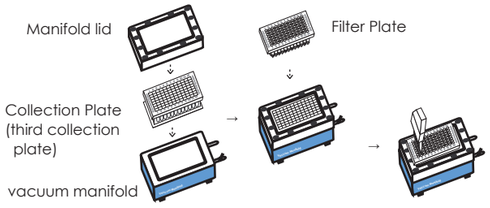
• Transfer upper clarified lysate to a clean Collection Plate (second collection plate) →  • Add 70 % ethanol →  • Mix by pipetting

↙ or ↘

• STEP 4. Bind RNA to Filter Plate:

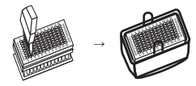
centrifuge protocol	vacuum protocol
• Combind the plates. • Transfer the sample mixture to Filter plate. • Centrifuge at 4,500 – 6,000 x g for 2 min.	• Fix Plates to manifold. • Transfer the sample mixture to Filter plate. • Apply 10 inches Hg vacuum until the wells have emptied.





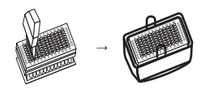
• (*Optional*) : Digest DNA by DNase I

• A1. Add Wash Buffer 1. Centrifuge at 4,500 – 6,000 x g for 5 min. • A2. Add DNase I mixture. Stand at R.T for 15 min. • A3. Add Wash Buffer 1. Centrifuge at 4,500 – 6,000 x g for 2 min. • **A4. Proceed to STEP 6.**	• B1. Add Wash Buffer 1. Apply vacuum at 10 inches Hg. • B2. Add DNase I mixture. Stand at R.T for 15 min. • B3. Add Wash Buffer 1. Apply vacuum at 10 inches Hg. • **B4. Proceed to STEP 6.**



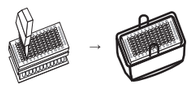
• STEP 5. Wash Filter Plate with Wash Buffer 1

• Add Wash Buffer 1. Centrifuge at 4,500 – 6,000 x g for 2 min.	• Add Wash Buffer 1. Apply vacuum at 10 inches Hg.



• STEP 6& 7. Wash Filter Plate with Wash Buffer 2

• STEP 6: Add Wash Buffer 2. Centrifuge at 5,600 - 6,000 x g for 2 min • STEP 7: Add Wash Buffer 2. Centrifuge at 5,600 - 6,000 x g for **15 min**	• STEP 6 : Add Wash Buffer 2. Apply vacuum at 10 inches Hg. • STEP 7: Add Wash Buffer 2 Apply vacuumat at 10 inches Hg for **10 min**.

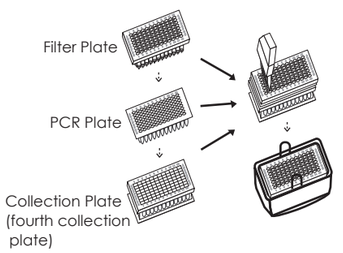


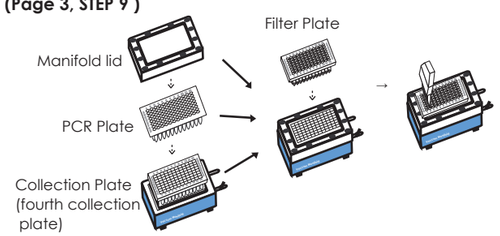
• STEP 8. Dry the membranes of Filter Plate:

• Stand the Filter plate on a clean paper towel at room temperature for 10 min.	• Tap the Filter Plate tips on paper towel • Return the Filter Plate and the Collection Plate to the manifold. • Apply maximum vacuum for an additional 10 min.

• STEP 9. RNA Elution:

• Add RNase-free Water to the Filter Plate. Stand for 3 min. • Centrifuge to elute RNA.	• Add RNase-free Water to the Filter Plate. Stand for 3 min. • Close the manifold volve. Turn on the vacuum source to build up a vacuum to 15 inches Hg. • Open the manifold volve to apply vacuum to elute DNA/ RNA. **Alternative: If the consistent volume of elutes are recommend use centrifuge protocol to proceed this elution step. (Page 3, STEP 9)**





Protocol: (centrifugation processing)

Please Read Important Notes Before Starting The Following Steps.

Required hardware
Centrifuge equiment capable of 5,600 ~ 6,000 X g with a swing -bucket rotor and the adaptor for 96-well plate

STEP 1. Sample preparation and lysis

For animal cells:

- Transfer up to 1 x 10^7 cells to each well of a Collection Plate (provided, 96-well 2 ml plate; first collection plate). Centrifuge the plate at 500 x g, 4 °C for 5 min. Remove the supernatant.
- Add 450 µl of Lysis Buffer and 4.5 µl of ß-Mercaptoethanol. Pipet up and down to resuspend the cells completely.
- Incubate the sample mixture at room temperature for 5 min.

For animal tissues :

- Transfer up 50 mg tissue to each well of a Collection Plate (provided, first collection plate).
- Add 450 µl of Lysis Buffer and 4.5 µl of ß-Mercaptoethanol.
- Disrupt the sample with a appropriate homogenizer.
- Incubate the sample mixture at room temperature for 5 min.

STEP 2. Clarify lysate

- Seal the Adhesive Film on the Collection Plate. Place the plate in a rotor bucket and centrifuge at 5,600 – 6,000 x g for 10 min.

STEP 3. Adjust binding condition

- Transfer 350 µl of the upper clarified lysate to each well of a clean Collection Plate (provided, second collection plate).
  -- Note: Avoid to pipet any debris and pellet when transfering the supernatant.
- Add 350 µl of 70 % RNase-free ethanol to each well and mix by pipetting.
  -- Note: make sure that ethanol have been mixed completely.

STEP 4. RNA Binding

- Place a Filter Plate (provided, 96-Well nucleic acid binding plate) on a clean Collection Plate (provided, third collection plate).
- Transfer the sample mixture to each well of the Filter Plate and discard the Collection Plate (second collection plate).
- Place the plates in a rotor bucket and centrifuge at 5,600 – 6,000 x g for 2 min.
- Discard the flow-through and return the Filter Plate to the Collection Plate.

(Optional STEP) : Digest DNA by DNase I

Follow the steps from A1 ~ A4 to eliminate DNA. Otherwise, proceed STEP 5 directly.

- **A1**. Add 250 µl of Wash Buffer 1 (ethanol added) to each well of the Filter Plate. Place the plates in a rotor bucket and centrifuge at 5,600 – 6,000 x g for 5 min. Discard the flow-through and return the Filter Plate to the Collection Plate.
- **A2**. Add 60 µl of RNase-free DNase I solution (0.5U/ul, not provided) to each well's membrane of the Filter Plate. Stand the plates for 15 min at room temperature. **Do not centrifuge after incubation, proceed step A3 directly.**
- **A3**. Add 250 µl of Wash Buffer 1 to the to each well of the Filter Plate. Place the plates in a rotor bucket and centrifuge at 5,600 – 6,000 x g for 2 min. Discard the flow-through and return the Filter Plate to the Collection Plate.
- **A4**. **After DNase I treatment, proceed STEP 6.**

STEP 5. Wash the Filter Plate with Wash Buffer 1

- Add 500 µl of Wash Buffer 1 (ethanol added) to each well of the Filter Plate.
- Place the combined plate in a rotor bucket and centrifuge at 5,600 – 6,000 x g for 2 min.
- Discard the flow-through and return the Filter Plate to the Collection Plate.

STEP 6. Wash the Filter Plate with Wash Buffer 2

- Add 500 µl of Wash Buffer 2 (ethanol added) to each well of the Filter Plate.
- Place the combined plate in a rotor bucket and centrifuge at 5,600 – 6,000 x g for 2 min.
- Discard the flow-through and return the Filter Plate to the Collection Plate.

STEP 7. Wash the Filter Plate again with Wash Buffer 2

- Add 500 µl of Wash Buffer 2 (ethanol added) to each well of the Filter Plate.
- Centrifuge at 5,600 – 6,000 x g for 15 min.
- Discard the flow-through and the Collection Plate (third collection plate).

STEP 8. Dry the membranes of Filter Plate

- Place the Filter Plate on top of a clean paper towel (not provided) and stand at room temperature for 10 min.

STEP 9. RNA Elution

- Place a Elution Plate (provided, 96-Well PCR plate) on top of a clean Collection Plate (provided, fourth collection plate) then place the Filter Plate on the Elution plate. (top: Filter Plate, middle: 96-well PCR Plate, bottom: Collection Plate)
- Add 50 ~ 75 µl of RNase-free Water to the membrane center of the Filter Plate. Stand for 3 min.
  -- Important Step! For effective elution, make sure that RNase-free water is dispensed on the membrane center and is absorbed completely.
  -- Important : Do not elute the DNA/ RNA using RNase-free water less than suggested volume (< 50 µl). It will lower the RNA yield.
- Place the plates in a rotor bucket and centrifuge at 5,600 – 6,000 x g for 5 min to elute RNA.
- Seal the Adhesive Film and store the RNA at -70 °C.

Protocol: (Vacuum processing)

Please Read Important Notes Before Starting The Following Steps.

Required hardware
Vacuun manifold for 96-well plate and vaccum source reached to -15 inches Hg
Alternative: If using centrifugation for Elution Step (STEP 8), a centrifuge equiment is required, capable of 5,600 ~ 6,000 X g, with a swing -bucket rotor and the adaptor for 96-well plate.

STEP 1. Sample preparation and lysis

For animal cells:

- Transfer up to 1 x 10^7 cells to each well of a Collection Plate (provided, 96-well 2 ml plate; first collection plate). Centrifuge the plate at 500 x g, 4 °C for 5 min. Remove the supernatant.
- Add 450 µl of Lysis Buffer and 4.5 µl of ß-Mercaptoethanol. Pipet up and down to resuspend the cells completely.
- Incubate the sample mixture at room temperature for 5 min.

For animal tissues :

- Transfer up 50 mg tissue to each well of a Collection Plate (provided, first collection plate).
- Add 450 µl of Lysis Buffer and 4.5 µl of ß-Mercaptoethanol.
- Disrupt the sample with a appropriate homogenizer.
- Incubate the sample mixture at room temperature for 5 min.

STEP 2. Clarify lysate

- Seal the Adhesive Film on the Collection Plate. Place the plate in a rotor bucket and centrifuge at 5,600 – 6,000 x g for 10 min.

STEP 3. Adjust binding condition

- Transfer 350 µl of the upper clarified lysate to each well of a clean Collection Plate (provided, second collection plate).
  -- Note: Avoid to pipet any debris and pellet when transfering the supernatant.
- Add 350 µl of 70 % RNase-free ethanol to each well and mix by pipetting.
  -- Note: make sure that ethanol have been mixed completely.

STEP 4. RNA Binding

- Fix a clean Collection Plate (provided, third collection plate) on the rack of vacuum manifold and cover the manifold lid. Place a Filter Plate (provided, 96-Well nucleic acid binding plate) on top of the Collection Plate.
- Transfer the sample mixture to the Filter Plate and discard the Collection Plate (second collection plate).
- Apply vacuum at 10 inches Hg until the wells have emptied.
- Discard the flow-through and return the Filter Plate and the Collection Plate to the manifold.

(Optional STEP) : Digest DNA by DNase I

Follow the steps from B1 ~ B4 to eliminate DNA. Otherwise, proceed STEP 5 directly.

- **B1**. Add 250 µl of Wash Buffer 1 (ethanol added) to each well of the Filter Plate. Apply vacuum at 10 inches Hg for 2 min. Discard the flow-through and return the Filter Plate to the manifold.
- **B2**. Add 60 µl of RNase-free DNase I solution (0.5U/ul, not provided) to each well's membrane of the Filter Plate. Stand the plate for 15 min at room temperature. **Do not centrifuge after incubation, proceed step B3 directly.**
- **B3**. Add 250 µl of Wash Buffer 1 to the to each well of the Filter Plate. Apply vacuum at 10 inches Hg until the wells have emptied. Discard the flow-through and return the Filter Plate to the manifold.
- **B4**. **After DNase I treatment, proceed STEP 6.**

STEP 5. Wash the Filter Plate with Wash Buffer 1

- Add 500 µl of Wash Buffer 1 (ethanol added) to each well of the Filter Plate.
- Apply vacuum at 10 inches Hg until the wells have emptied.
- Discard the flow-through and return the Filter Plate and the Collection Plate to the manifold.

STEP 6. Wash the Filter Plate with Wash Buffer 2

- Add 500 µl of Wash Buffer 2 (ethanol added) to each well of the Filter Plate.
- Apply vacuum at 10 inches Hg until the wells have emptied.
- Discard the flow-through and return the Filter Plate and the Collection Plate to the manifold.

STEP 7. Wash the Filter Plate again with Wash Buffer 2

- Add 500 µl of Wash Buffer 2 (ethanol added) to each well of the Filter Plate.
- Apply vacuum at 10 inches Hg for **10 min**.
- Discard the flow-through and return the Collection Plate to the manifold.

STEP 8. Dry the membranes of Filter Plate

- Gently tap the tips of the Filter Plate on a clean paper towel to remove residual liquid.
- Return the Filter Plate to the Collection Plate fixed in the manifold.
- Apply vacuum for an addition 10 min.
- Discard the flow-through and the Collection Plate (third plate).

STEP 9. RNA Elution

- Place a Elution Plate (provided, 96-Well PCR plate) on top of a clean Collection Plate (provided, fourth collection plate) and fix plates on the rack of manifold. Cover the manifold lid and place the Filter Plate on the Elution Plate. (top: Filter Plate, middle: 96-well PCR Plate, bottom: Collection Plate)
- Add 50 ~ 75 µl of RNase-free Water to the membrane center of the Filter Plate. Stand for 3 min.
  -- Important Step! For effective elution, make sure that RNase-free water is dispensed on the membrane center and is absorbed completely.
  -- Important : Do not elute the RNA using RNase-free water less than suggested volume (< 50 µl). It will lower the RNA yield.
- Close the manifold volve. Turn on the vacuum source to build up a vacuum to 15 inches Hg.
- Open the manifold volve to apply vacuum to elute RNA.
- Seal the Adhesive Film and store the RNA at -70 °C.

Alternative: If the consistent volume of elutes are recommend use centrifuge protocol to proceed this elution step. (Page 3, STEP 9)