User Manual

FavorPrep™ 96-Well Total RNA Kit

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

For Research Use Only

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 Binding 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 pcs

■,▲,♦,●: Add ethanol (RNase-free, 96~100%) to Wash Buffer 1 and Wash Buffer 2 when first use.

Storage:

All components of FavorPrep™ 96-Well Total RNA Kit should be stored at room temperature (15 ~ 25 °C).

Quality control

The quality of FavorPrep™ 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 (96-well plate, silica membrane)
Sample size: animal cells, up to 1 x 10^7 / preparation
animal tissue up to 50 mg tissues / preparation
Processing: vacuum or centrifugation
Operation time: < within 60 min/ 96 preparations
RNA Binding capacity: up to 75 µg/ well
Elution volume: 50 ~ 75 µl
Downstream application: Real-time RT-PCR, cDNA synthesis, Northern blotting, primer extension and mRNA selection etc

Product description:

FavorPrep™ 96-well Tissue Total RNA Kit is designed for 96 wells high-throughput isolation of total RNA from animal cultured cells or animal tissues which offer a speedy method to purify total RNA and prevent the degradation of the RNA during the isolation procedure. The technology using a chaotropic salt buffer to lyses the cells, inactive the RNase and binds RNA (> 200 nt, e.g., 18S, 28S RNA, pri-miRNA) to the silica membranes of the Filter Plate. With optional on-column DNase I digestion for further DNA removal and membrane washed by 2 wash buffers. Then the highly pure RNA are eluted from the membrane in a low-ionic-strength buffer and are captured in an elution plate. This extracted total RNA can be used directly for the downstream applications such as Real-time RT-PCR, cDNA synthesis, Northern blotting, primer extension and mRNA selection, etc.

Important note:

1. Make sure the workstation is RNase-free when handling RNA.
2. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
3. Add ethanol (RNase-free, 96 ~ 100%) to Wash Buffer 1 and Wash Buffer 2 when first use.
4. Prepare working lysis buffer and working DNase I solution (for optional step: Digest DNA by DNase I) before starting the isolation procedure.
5. ***CAUTION:*** Lysis Buffers and Wash Buffer 1 contain guanidinium salts which can form highly reactive compounds when combined with bleach. ***DO NOT add bleach or acidic solutions directly to the preparation waste.***
6. ***CAUTION:*** **ß-mercaptoethanol (ß-Me) is hazardous to human health. perform the procedures involving ß-Me in a chemical fume hood.**
7. The eluted RNA should immediately be kept on ice. For long-term storage, freeze it at -70°C.

Additional materials required

For All Protocol:
- Pipets and pipet tips, sterile (nuclease-free)
- ß-mercaptoethanol (ß-Me)
- 96 ~ 100% RNase-free ethanol (for preparation of Wash Buffer).
- 70% RNase-free ethanol
- Crushed ice
- RNase-free DNase I and DNase I reaction buffer

For vacuum processing:
- A centrifugator is required for the clarification of lysate and for the alternative of elution step, capable of 5,600 ~ 6,000 X g, with a swing -bucket rotor and the adaptor for 96-well plates.
- A vacuum manifold for 96-well plate and a vacuum source reached to -12 inches Hg are required.

For centrifuge processing:
- A centrifugator is required, capable of 5,600 ~ 6,000 X g, with a swing-bucket rotor and the adaptor for 96-well plates.

Preparation of working buffers:

1. **Working Lysis Buffer**
   Add ß-mercaptoethanol (ß-Me) to Lysis Buffer, and mix well to make a 1% ß-Me-Lysis Buffer. For example, add 10 µl of ß-Me to 1 ml of Lysis Buffer.

2. **Working Wash Buffer**
   Add RNase-free ethanol to Wash Buffer 1 and Wash Buffer 2 when first use. Store the buffers at room temperature (15 ~ 25 °C).

Cat. No.	FATRE 96001	FATRE 96002, FATRE 96004
Ethanol for Wash Buffer 1	■ 20 ml	▲40 ml
Ethanol for Wash Buffer 2	♦100 ml	●200 ml

3. **Working DNase I reaction solution (*for Optional Step*)**
   Dilute RNase-free DNase I in DNase I Reaction buffer (1M NaCl, 10 mM $MnCl_2$, 20 mM Tris-HCl, pH 7.0) to final conc. 0.5U/µl. And stored at 4 °C before use.

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

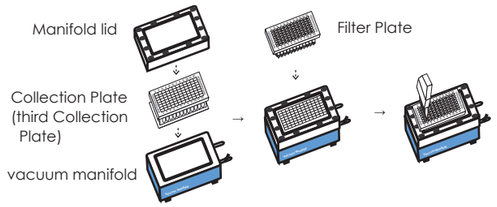
● STEP 3. Adjust binding condition:

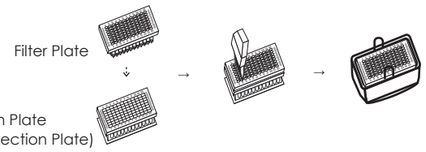
• Transfer upper clarified lysate to a clean Collection Plate (second Collection Plate) → • Add 70 % ethanol → • Mix by pipetting

↙ or ↘

● STEP 4. Bind RNA to Filter Plate:

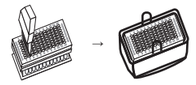
Vacuum processing	Centrifuge processing
• Transfer the sample mixture to Filter plate. • Apply -12 inches Hg vacuum until the wells have emptied.	• Transfer the sample mixture to Filter plate. • Centrifuge at 4,500 ~ 6,000 x g for 2 min.





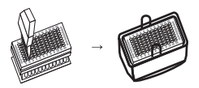
● (*Optional*) : Digest DNA by DNase I

• A1. Add Wash Buffer 1. Apply vacuum at -12 inches Hg. • A2. Add DNase I mixture. Stand at R.T for 15 min. • A3. Add Wash Buffer 1. Apply vacuum at -12 inches Hg. • **A4. Proceed to STEP 6.**	• B1. Add Wash Buffer 1. Centrifuge at 4,500 ~ 6,000 x g for **5 min**. • B2. Add DNase I mixture. Stand at R.T for 15 min. • B3. Add Wash Buffer 1. Centrifuge at 4,500 ~ 6,000 x g for 2 min. • **B4. Proceed to STEP 6.**



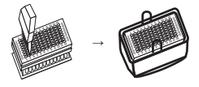
● STEP 5. Wash the Filter Plate with Wash Buffer 1

• Add Wash Buffer 1. Apply vacuum at -12 inches Hg.	• Add Wash Buffer 1. Centrifuge at 4,500 ~ 6,000 x g for 2 min.



● STEP 6 & 7. Wash the Filter Plate with Wash Buffer 2

• **STEP 6 & 7:** Add Wash Buffer 2. Apply vacuum at -12 inches Hg for 2 min.	• **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 **10 min**

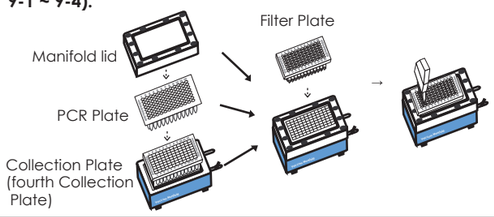


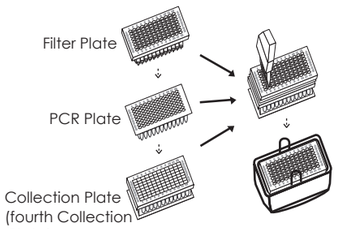
● STEP 8. Dry the membranes of Filter Plate:

• Tap the Filter Plate tips on paper towel • Return the Filter Plate and the Collection Plate to the manifold. • Apply vacuum at -12 inches Hg for an additional **10 min**.	• Stand the Filter plate on a clean paper towel at room temperature for **5 min**.

● STEP 9. RNA Elution:

• Add RNase-free Water to the Filter Plate. Stand for 3 min. • Close the manifold valve. Turn on the vacuum source to build up a vacuum to -12 inches Hg. • Open the manifold valve to apply vacuum to elute RNA. **Alternative : If the consistent volume of elutes are recommend, use centrifuge protocol to proceed this elution step. (Page 3, STEP 9-1 ~ 9-4).**	• Add RNase-free Water to the Filter Plate. Stand for 3 min. • Centrifuge to elute RNA.





Sample amount and typical yield

Sample	Recommended amount of sample used	Typical yield (µg)
Animal cells (up to 1 x 10^7)	HeLa, 1 x 10^6 cells	10
High yield Tissue (Mouse)(up to 20 mg)	Liver, 10 mg	35
	Spleen, 10 mg	45
Low yield Tissue (Mouse)(up to 50 mg)	Embryo, 10 mg	10
	Heart, 10 mg	7.5
	Brain, 10 mg	7.5
	Kidney, 10 mg	20
	Lung, 10 mg	10
	Intestine, 10 mg	15

Safety Information:

1. Lysis Buffer and Wash Buffer 1 provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. ***CAUTION:*** Lysis Buffers and Wash Buffer1 contain guanidinium salts which can form highly reactive compounds when combined with bleach. ***DO NOT add bleach or acidic solutions directly to the preparation waste.***

Kit Component: **Lysis Buffer**

Hazard contents
Guanidinium thiocyanate
CAS-No. 593-84-0
EC-No. 209-812-1

Danger

Hazard statement(s)

H302 + H312 + H332	Harmful if swallowed, in contact with skin or if inhaled.
H314	Causes severe skin burns and eye damage.
H412	Harmful to aquatic life with long lasting effects.

Precautionary statement(s)

P260	Do not breathe dust/ fume/ gas/ mist/ vapours/ spray.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P301 + P312 + P330	IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. Rinse mouth.
P303 + P361 + P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
P304 + P340 + P310	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Kit Component: **Wash Buffer 1**

Hazard contents
Guanidine hydrochloride
CAS-No. 50-01-1
EC-No. 200-002-3

Warning

Hazard statement(s)

H302 + H332	Harmful if swallowed or if inhaled.
H315	Causes skin irritation.
H319	Causes serious eye irritation.

Precautionary statement(s)

P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P301 + P312 + P330	IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. Rinse mouth.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Protocol: Vacuum processing

Please read Important Notes and Additional Materials Required before starting the following steps.

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 (ß-Me added). Pipet up and down to resuspend the cells completely.
- Incubate the sample mixture at room temperature for 5 min.

For animal tissues:
- Transfer up to 50 mg tissue to each well of a Collection Plate. (provided, 96-well 2 ml plate; first Collection Plate).
- Add 450 µl of Lysis Buffer (ß-Me added).
- Disrupt the sample with a appropriate homogenizer.
- Incubate the sample mixture at room temperature for 5 min.

STEP 2. Clarify lysate
- Seal with 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 transferring the supernatant.
- Add 350 µl of 70% RNase-free ethanol to each well and mix by pipetting.
  -- Note: make sure that ethanol mixed with lysate 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 RNA binding plate) on top of the third Collection Plate.
- Transfer the sample mixture to the Filter Plate and discard the second Collection Plate).
- Apply vacuum at -12 inches Hg until the wells have emptied.
- Release vacuum from the manifold.
- Discard the flow-through. Return the Filter Plate and the third Collection Plate back to the manifold.

(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. Apply vacuum at -12 inches Hg for 2 min. **Release vacuum from the manifold.**
- **A2**. Add 60 µl of DNase I reaction mixture (0.5 U/ul, not provided) to each well's membrane of the Filter Plate. Stand the plate for 15 min at room temperature. **Do not vacuum after incubation. Proceed step A3 directly.**
- **A3**. Add 250 µl of Wash Buffer 1 to each well of the Filter Plate. Apply vacuum at -12 inches Hg until the wells have emptied. Release vacuum from the manifold. Discard the flow-through. Return the Filter Plate and third Collection Plate back to the manifold.
- **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.
- Apply vacuum at -12 inches Hg until the wells have emptied.
- Release vacuum from the manifold.
- Discard the flow-through. Return the Filter Plate and the third Collection Plate back 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 -12 inches Hg for 2 min.
- Release vacuum from the manifold.
- Discard the flow-through. Return the Filter Plate and the third Collection Plate back to the manifold.

STEP 7. Wash the Filter Plate "again" with Wash Buffer 2
- Repeat Step 6

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 third Collection Plate fixed in the manifold.
- Apply vacuum at -12 inches Hg for an addition **10 min**.
- Release vacuum from the manifold.
- Discard the flow-through and the third Collection Plate.

STEP 9. RNA Elution
Alternative: If the consistent volume of eluates are recommended, use "centrifuge processing step 9-1 ~ 9-4", to proceed this elution.
- Place an 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: fourth Collection Plate)
- Add 50 ~ 75 µl of RNase-free Water to the membrane center of the Filter Plate. Stand for 3 min.
  -- Note! The eluates averaged about 25 µl less than the adding volume of elution buffers. For example, adding 50 µl of RNase-free water will recover ~25 µl of eluate.
  -- Note! Do not use RNase-free water less than the suggested volume (< 50 µl). It will lower the RNA yield.
  -- Note! For effective elution, make sure that RNase-free water is dispensed on the membrane center and is absorbed completely.
- Close the manifold valve. Turn on the vacuum source to build up a vacuum to -12 inches Hg.
- Open the manifold volve to apply vacuum to elute RNA.
- Release vacuum from the manifold.
- Take out the Elution Plate (96-well PCR plate) and seal with an Adhesive Film (provided). Store the RNA at -70°C before use.

Protocol: Centrifuge processing

Please read Important Notes and Additional Materials Required before starting the following steps.

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 (ß-Me added). Pipet up and down to resuspend the cells completely.
- Incubate the sample mixture at room temperature for 5 min.

For animal tissues:
- Transfer up to 50 mg tissue to each well of a Collection Plate. (provided, 96-well 2 ml plate; first Collection Plate).
- Add 450 µl of Lysis Buffer (ß-Me added).
- Disrupt the sample with a appropriate homogenizer.
- Incubate the sample mixture at room temperature for 5 min.

STEP 2. Clarify lysate
- Seal with 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 transferring the supernatant.
- Add 350 µl of 70 % RNase-free ethanol to each well and mix by pipetting.
  -- Note: make sure that ethanol mixed with lysate completely.

STEP 4. RNA Binding
- Place a Filter Plate (provided, 96-Well RNA 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 second Collection Plate.
- Place the combined plates (Filter Plate + the third Collection 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 third Collection Plate.

(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. Place the combined 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 back to the third Collection Plate.
- **B2**. Add 60 µl of DNase I solution (0.5 U/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 B3 directly.**
- **B3**. Add 250 µl of Wash Buffer 1 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 third Collection Plate.
- **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.
- 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 back to the third 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 third 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.
- Place the combined plate in a rotor bucket and centrifuge at 5,600 ~ 6,000 x g for **10 min**.
- Discard the flow-through and the 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 **5 min**.

STEP 9. RNA Elution
9-1. Place an 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: fourth Collection Plate)
9-2. Add 50 ~ 75 µl of RNase-free Water to the membrane center of the Filter Plate. Stand for 3 min.
-- Note! The eluates averaged about 25 µl less than the adding volume of elution buffers. For example, adding 50 µl of RNase-free water will recover ~ 25 µl of eluate.
-- Note! Do not use RNase-free water less than the suggested volume (< 50 µl). It will lower the RNA yield.
-- Note! For effective elution, make sure that RNase-free water is dispensed on the membrane center and is absorbed completely.
9-3. Place the combined plates in a rotor bucket and centrifuge at 5,600 ~ 6,000 x g for 5 min to elute RNA.
9-4. Take out the Elution Plate (96-well PCR plate) and seal with an Adhesive Film (provided). Store the RNA at -70°C before use.