User Manual

# FavorPrep™ Tissue Total RNA Extraction 96-Well 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) | FATR107A (1 plate) | FATR107B (2 plates) | FATR107C (4 plates) |
|---|---|---|---|
| FARB Buffer | 60 ml | 120 ml | 120 ml × 2 |
| Wash Buffer 1 (Concentrate) | 55 ml ■ | 110 ml ▲ | 110 ml × 2 ▲ |
| Wash Buffer 2 (Concentrate) | 25 ml ♦ | 50 ml ● | 50 ml × 2 ● |
| RNase-Free Water | 15 ml | 30 ml | 30 ml × 2 |
| Filter Plates (96-Well RNA Binding plate) | 1 plate | 2 plates | 4 plates |
| Collection Plates (96-Well 2 ml Plate) | 4 plates | 8 plates | 16 plates |
| Elution Plates (96-Well PCR plate) | 1 plate | 2 plates | 4 plates |
| Adhesive Films | 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™ Tissue Total RNA Extraction 96-Well Kit should be stored at room temperature (15~25 °C).

## Quality control

The quality of FavorPrep™ Tissue Total RNA Extraction 96-Well 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 $1\times10^7$/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™ Tissue Total RNA Extraction 96-Well 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:*** FARB 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.
8. Ensure plates are tightly sealed with **Adhesive Film** to prevent sample loss or cross-well contamination. Never reuse adhesive film.

## 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 2).
- 70% RNase-free ethanol
- Crushed ice
- RNase-free DNase I and DNase I reaction buffer
- 96-Well PCR Rack

***For vacuum processing:***
- A centrifuge capable of reaching a minimum speed of 5,600~6,000 xg with a microplate swinging-bucket rotor (capable of accommodating an 8.0 cm plate stack).
- A vacuun manifold for 96-well plate and a vaccum source reached to -12 inches Hg are required.

***For centrifuge processing:***
- A centrifuge capable of reaching a minimum speed of 5,600~6,000 xg with a microplate swinging-bucket rotor (capable of accommodating an 8.0 cm plate stack).

## Preparation of working buffers:

1. **Working lysis Buffer**
Add ß-mercaptoethanol (ß-Me) to FARB Buffer, and mix well to make a 1% ß-Me-FARB Buffer. For example, add 10 µl of ß-Me to 1 ml of FARB 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. | FATR107A | FATR107B, FATR107C |
|---|---|---|
| Ethanol for Wash Buffer 1 | ■ 20 ml | ▲40 ml |
| Ethanol for Wash Buffer 2 | ♦100 ml | ●200 ml |

3. **Preparation of "RNase-free" DNase I reaction solution for Optional Step, On-Column DNase I Digestion.**
For each reaction, prepare 60 µl of RNase-free DNase I solution (0.25 U/µl). Prepare a 10× DNase I reaction buffer containing 1 M NaCl, 10 mM $MnCl_2$ or $MgCl_2$, and 20 mM Tris-HCl (pH 7.0 at 25°C). Dilute this buffer to a 1× working concentration before use. Use the 1× buffer to dilute the DNase I enzyme to a final concentration of 0.25 U/µl. Alternatively, use the ready-to-use **FavorPrep™ DNase I Solution (Cat. No. FADI2093)** to simplify preparation.



## Brief procedure:

**● STEP 1. Sample preparation and lysis**
• Collect samples in a Collection Plate (first Collection Plate) → • Add FARB Buffer → • Disrupt the samples → • Stand at room temperature for 5 mins

**● STEP 2. Clarify lysate**
• Seal with Adhesive Film.
• Centrifuge at 5,600~6,000 ×g for 10 mins

**● STEP 3. Adjust binding condition:**

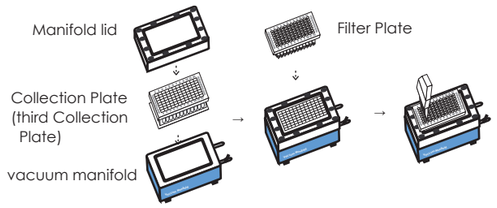
• Transfer upper clarified lysate to a clean Collection Plate (second Collection Plate) → • Add RNase-free 70% ethanol → • Mix by pipetting

↙ or ↘

**● STEP 4. Bind RNA to Filter Plate:**

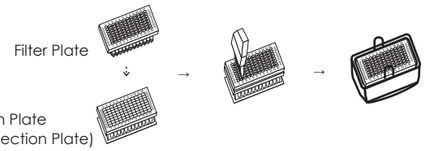
**Vacuum processing**

• Transfer the sample mixture to Filter plate.
• Apply -12 inches Hg vacuum until the wells have emptied.




**Centrifuge processing**

• Transfer the sample mixture to Filter plate.
• Centrifuge at 4,500~6,000 ×g for 2 mins.




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

• A1. Add Wash Buffer 1. Apply vacuum at -12 inches Hg.
• A2. Add RNase-free 70% ethanol. Apply vacuum at -12 inches Hg.
• A3. Add DNase I mixture. Stand at R.T for 15 min.
• A4. Add Wash Buffer 1. Apply vacuum at -12 inches Hg.
• **A5. Proceed to STEP 6.**



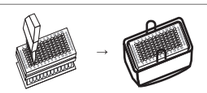
• B1. Add Wash Buffer 1. Centrifuge at 4,500~6,000 ×g for **5 mins**.
• B2. Add RNase-free 70% ethanol. at 4,500~6,000 ×g for **5 mins**.
• B3. Add DNase I mixture. Stand at R.T for 15 mins.
• B4. Add Wash Buffer 1. Centrifuge at 4,500~6,000 ×g for 2 mins.
• **B5. Proceed to STEP 6.**



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

• Add Wash Buffer 1. Apply vacuum at -12 inches Hg.

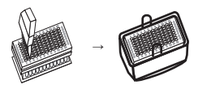
• Add Wash Buffer 1. Centrifuge at 4,500~6,000 ×g for 2 mins.



**● 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 mins.

• **STEP 6:**
Add Wash Buffer 2. Centrifuge at 5,600~6,000 ×g for 2 mins
• **STEP 7:**
Add Wash Buffer 2. Centrifuge at 5,600~6,000 ×g for **10 mins**



**● STEP 8. Dry the membranes of Filter Plate:**

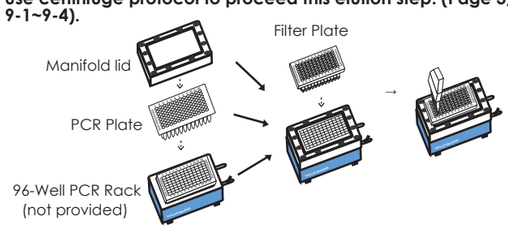
• Tap the Filter Plate tips on paper towel
• Return the Filter Plate and the Collection Plate to the manifold.
• Apply vacuum at -12 inches Hg for an additional **10 mins**.

• Stand the Filter plate on a clean paper towel at room temperature for **5 mins**.

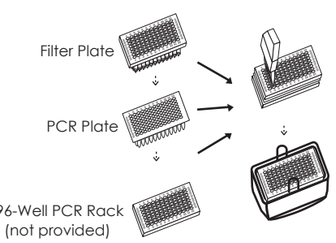
**● STEP 9. RNA Elution:**

• Add RNase-Free Water to the Filter Plate. Stand for 3 mins.
• 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 mins.
• Centrifuge to elute RNA.



## Sample amount and typical yield

| Sample | Recommended amount of sample used | Typical yield (µg) |
|---|---|---|
| Animal cells (up to $1\times10^7$) | HeLa, $1\times10^6$ 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. FARB Buffer and Wash Buffer 1 provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. ***CAUTION:*** FARB 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: **FARB 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 $1\times10^7$ cells to each well of a Collection Plate. (provided, 96-well 2 ml plate; first Collection Plate). Centrifuge the plate at 500 ×g, 4°C for 5 mins. Remove the supernatant.
- Add 450 µl of FARB Buffer (ß-Me added). Pipet up and down to resuspend the cells completely.
- Incubate the sample mixture at room temperature for 5 mins.

**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 FARB 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 ×g for 10 mins.

**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 RNase-free 70% 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 Collection Plate (third).
- Place the Filter Plate and a clean Collection Plate (provided, fourth Collection Plate) 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 mins. **Release vacuum from the manifold.**
- **A2**. Add 750 µl of RNase-free 70% ethanol to each well of the Filter Plate. Apply vacuum at -12 inches Hg for 2 mins. **Release vacuum from the manifold.**
- **A3**. Add 60 µl of DNase I reaction mixture (0.25 U/µl, not provided) to each well's membrane of the Filter Plate. Stand the plate for 15 mins at room temperature. **Do not vacuum after incubation. Proceed step A4 directly.**
- **A4**. 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 Collection Plate back to the manifold.
- **A5**. **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 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 mins.



- Release vacuum from the manifold.
- Discard the flow-through. Return the Filter Plate and the 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 Collection Plate fixed in the manifold.
- Apply vacuum at -12 inches Hg for an addition **10 mins**.
- Release vacuum from the manifold.
- Discard the 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 the 96-Well PCR Rack (not provided) and fix plate onto manifold. Cover the manifold lid and place the Filter Plate on the Elution Plate (top: Filter Plate; middle: 96-Well PCR Plate; bottom: 96-Well PCR Rack).
- Add 50~75 µl of RNase-Free Water to the membrane center of the Filter Plate. Stand for 3 mins.
  **-- 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 $1\times10^7$ cells to each well of a Collection Plate. (provided, 96-well 2 ml plate; first Collection Plate). Centrifuge the plate at 500 ×g, 4°C for 5 mins. Remove the supernatant.
- Add 450 µl of FARB Buffer (ß-Me added). Pipet up and down to resuspend the cells completely.
- Incubate the sample mixture at room temperature for 5 mins.

**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 FARB Buffer (ß-Me added).
- Disrupt the sample with a appropriate homogenizer.
- Incubate the sample mixture at room temperature for 5 mins.

**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 ×g for 10 mins.

**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 RNase-free 70% 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 ×g for 2 mins.
- Discard the Collection Plate.
- Place the Filter Plate on a clean Collection Plate (provided, fourth 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 ×g for 5 mins. Discard the flow-through and return the Filter Plate back to the Collection Plate.
- **B2**. Add 750 µl of RNase-free 70% ethanol to each well of the Filter Plate. Place the combined plates in a rotor bucket and centrifuge at 5,600~6,000 ×g for 5 mins. Discard the flow-through and return the Filter Plate back to the Collection Plate.
- **B3**. Add 60 µl of DNase I solution (0.25 U/µl, not provided) to each well's membrane of the Filter Plate. Stand the plates for 15 mins at room temperature. **Do not centrifuge after incubation. Proceed step B4 directly.**
- **B4**. 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 ×g for 2 mins. Discard the flow-through and return the Filter Plate to the Collection Plate.
- **B5**. **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 ×g for 2 mins.
- Discard the flow-through and return the Filter Plate back 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 ×g for 2 mins.
- 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.
- Place the combined plate in a rotor bucket and centrifuge at 5,600~6,000 ×g for **10 mins**.
- Discard the 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 mins**.

**STEP 9. RNA Elution**

9-1. Place the combined Filter Plate and Elution Plate (provided, 96-Well PCR Plate) onto the 96-Well PCR Rack (not provided), forming a three-plate assembly in the following order: top – DNA Binding Plate; middle – Elution Plate; bottom – 96-Well PCR Rack.

9-2. Add 50~75 µl of RNase-Free Water to the membrane center of the Filter Plate. Stand for 3 mins.
**-- 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 ×g for 5 mins 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.