User Manual

FavorPrep™ Tissue Total RNA Mini Kit

-- For isolation RNA from animal cells, animal tissues, bacteria, yeast, paraffin fixed sample, fungi and for RNA clean-up

For Research Use Only

Kit Contents:

Cat. No:	FATRK 000-Mini (4 preps_sample)	FATRK 001 (50 preps)
FARB Buffer	1.5 ml × 2	25 ml
Wash Buffer 1	1.5 ml × 2	30 ml
Wash Buffer 2 ■ (concentrate	1.5 ml	15 ml
RNase-free Water	0.5 ml	6 ml
Filter Columns	4 pcs	50 pcs
FARB Mini Columns	4 pcs	50 pcs
Collection Tubes	8 pcs	100 pcs
Elution Tubes	4 pcs	50 pcs
Micropestles	4 pcs	50 pcs
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■, Adding Ethanol to the concentrate Wash Buffer 2. ***See Working Buffer Preparation***.

Cat. No:	FATRK 001-1 (100 preps)	FATRK 001-2 (300 preps)
FARB Buffer	45 ml	130 ml
Wash Buffer 1	60 ml	170 ml
Wash Buffer 2 ■ (concentrate)	35 ml	50 ml × 2
RNase-free Water	6 ml	8 ml × 2
Filter Columns	100 pcs	300 pcs
FARB Mini Columns	100 pcs	300 pcs
Collection Tubes	200 pcs	600 pcs
Elution Tubes	100 pcs	300 pcs
Micropestles	100 pcs	300 pcs
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■, Adding Ethanol to the concentrate Wash Buffer 2. ***see Working Buffer Preparation***.

Storage:

Kit components should be stored at room temperature (15~25°C).

Quality Control:

The quality of FavorPrep™ Tissue Total RNA Mini Kit is tested on a lot-to-lot basis according to ISO quality management system.

Specification:

Format/ Principle: spin column/silica membrane/chaotropic salt
Operation time: 30~60 minutes
Length of recovery nucleic acid : >200 bp
Column Binding capacity: 100 µg RNA/column
Elution volume: 30~50 µl
Column applicability: centrifugation and vacuum

Sample amount and yield:

Sample	Recommended amount of sample used		Yield (µg)
Animal cells (up to 5×10⁶)	NIH/3T3 HeLa COS-7 LMH	1×10⁶ cells	10 15 30 12
Animal Tissue (Mouse/rat) (up to 30 mg)	Embryo Heart Brain Kidney Liver Spleen Lung Thymus	10 mg	25 10 10 30 50 35 15 45
Bacteria	E. coli B. subtilis	1×10⁹ cells	60 40
Yeast (up to 5×10⁷)	S. cerevisiae	1×10⁷ cells	25

Important Note:

Notes for Buffers:

1. Make sure that the working environment is RNase-free.
2. Buffers provided by this kit containing irritants, wear gloves and lab coat for operation.
3. **Caution: ß-mercaptoethanol (ß-Me) is hazardous to human health. perform the procedures involving ß-Me in a chemical fume hood.**
4. The centrifuge force should be performed at ~18,000 x g.
5. The vacuum source should be reached to - 6 inches Hg.
6. Add ethanol (96~100%) to concentrate Wash Buffer 2 before use. see ***Working Buffer Preparation.***
7. For handling the buffers safely please read ***safety Information*** before starting the procedure.

2. Notes for centrifuging and vacuum:

- When using of vacuum to operate the RNA extraction, ensure that the tip of the column is fit into the shape of manifold adaptor, and the vacuum pressure being capable to reach to - 6 inches Hg.
- Units and values at same pressure (1 atm)

unit	value
atmosphere (atm)	1.000
millimeter of mercury (mmHg)	760.000
inches of mercury (inHg)	29.290
pascal (Pa)	101,325.000
kilopascal (KPa)	101.325
torr (torr)	760.000
pound per square inch (psi, 1bs/in²)	14.700

Materials and equipment provided by the user

For All Protocol:

- Pipets, pipet tips and centrifuge tubes (1.5 ml, 2.0 ml), sterile
- 96~100% ethanol (for preparation of Wash Buffer 2).

For centrifuge processing:

- A micro-centrifugator is capable of ~18,000 x g, with a rotor for 1.5 or 2.0 ml micro-centrifuge tube.

For vacuum processing:

- A micro-centrifugator is capable of ~18,000 x g, with a rotor for 1.5~2.0 ml micro-centrifuge tube.
- A vacuum manifold contains adaptors for Filter Columns and FARB Column. And the vacuum be capable to -6 inches Hg.

Working Buffer Preparations:

1. "■" **Preparation of Wash Buffer 2**
   Add RNase-free ethanol (96~100%) to the concentrate Wash Buffer 2 as the table indicated. And store the ethanol-added Wash Buffer 2 at 15~25°C.

Cat. No./ (preps)	Ethanol volume to **Wash Buffer 2**
FATRK 000-Mini/ (4 preps)	6 ml
FATRK 001/ (50 preps)	60 ml
FATRK 001-1/ (100 preps)	140 ml
FATRK 001-2/ (300 preps)	200 ml

2. **Preparation of "RNase-free" DNase I reaction solution for Optional Step, On-Column DNase I Digestion.**
   Preparation of a 0.5U/µl 50 µl/ prep"RNase-free" DNase I reaction solution **(Recommend):** add 3 µl of DNase I endoribonuclease (10U/µl) to 57 µl of a DNase I reaction buffer (1 M NaCl; 10 mM MnCl2 or MgCl2; 20 mM Tris-HCl, pH 7.0 at 25°C) to a final concentration of 0.5U/µl.

Safety Information:

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.***

Kit Component: **FARB Buffer**

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

GHS symbol  Warning

Hazard statement(s)
H302 + H312 + H332 Harmful if swallowed, in contact with skin or if inhaled.
H314 Causes severe skin burns and eye
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**

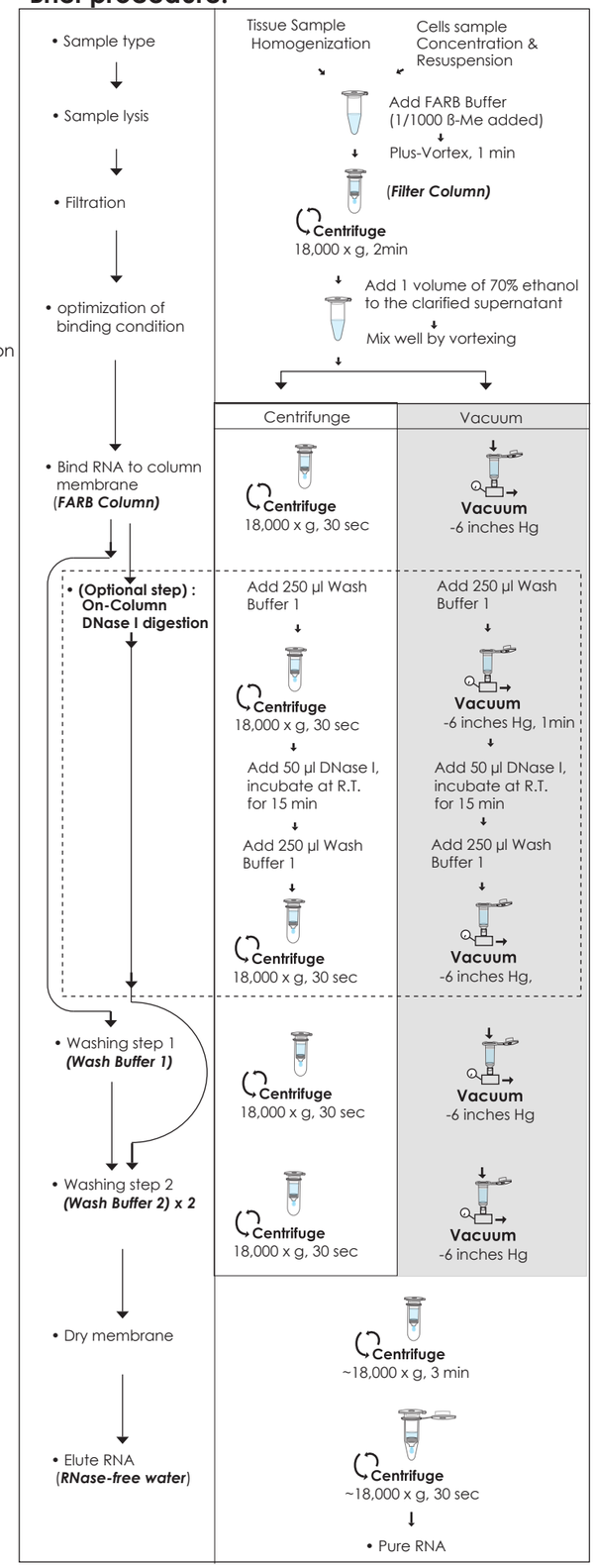
Hazard contents
Guanidine hydrochloride, 20~50%, CAS-No. 50-01-1

GHS symbol  Warning

Hazard statement(s)
H302 Harmful if swallowed.
H319 Causes serious eye irritation.

Precautionary statement(s)
P264 Wash … thoroughly after handling.
P280 Wear protective gloves/ protective clothing/ eye protection/ fac protection.
P301 + P312 + P330 IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. Rinse mouth.

Brief procedure:



General Protocol: Animal Cells

Please Read Important Notes Before Starting Following Steps.

Additional requirment: ß-Mercaptoethanol
70% RNase-free ethanol

1. Collect 1~5 ×10^6 cells by centrifuging at 300 x g for 5 min at 4°C. Remove all the supernatant. **-- Note!** Do not overload, too much sample will make cell lysis incompletely and lead to lower RNA yield and purity.

2. Add 350 µl of FARB Buffer and 3.5 µl of ß-Mercaptoethanol to the cell pellet. Vortex vigorously for 1 min to resuspend the cells completely. **-- Note:** If the clump is still visible after vortex, pipet the sample mixture up and down to break down the clump.

3. Place a Filter Column to a Collection Tube, and transfer the sample mixture to the Filter Column. Centrifuge at 18,000 x g for 2 min.

4. Transfer the clarified supernatant from the Collection Tube to a new microcentrifuge tube (not provided) and measure the volume of the supernatant. Discard the Filter Column and the Collection Tube. **-- Note:** Avoid to pipet any debris and pellet when transferring the supernatant.

5. Add 1 volume of 70% RNase-free ethanol and mix well by vortexing.

6. Place a FARB Mini Column to a Collection Tube and transfer the ethanol added sample mixture (including any precipitate) to the FARB Mini Column.
   (Centrifuge) Centrifuge at 18,000 x g for 30 sec. Discard the flow -through and return the FARB Mini Column back to the Collection Tube.
   (Vacuum) Apply vacuum at -6 inches Hg until the column have emptied. Switch off the vacuum and release vacuum from the manifold.

7. Optional step: On-Column DNase I digestion
   Follow the steps from 7a to eliminate genomic DNA contamination. **Otherwise, proceed to step 8 directly**.
   7a. Add 250 µl of Wash Buffer 1 to the FARB Mini Column.
   (Centrifuge) Centrifuge at 18,000 x g for 30 sec. Discard the flow -through and return the FARB Mini Column back to the Collection Tube.
   (Vacuum) Apply vacuum at -6 inches Hg for 1 min. Switch off the vacuum and release vacuum from the manifold.
   7b. Add 50 µl of RNase-free DNase I solution (0.5U/ul, not provided) to the membrane center of the FARB Mini Column. Place the column on the benchtop for 15 min.
   7c. Add 250 µl of Wash Buffer 1 to the FARB Mini Column.
   (Centrifuge) Centrifuge at 18,000 x g for 30 sec. Discard the flow -through and return the FARB Mini Column back to the Collection Tube.
   (Vacuum) Apply vacuum at -6 inches Hg until the column have emptied. Switch off the vacuum and release vacuum from the manifold.
   7d. **After DNase I treatment, proceed to step 9.**

8. Add 500 µl of Wash Buffer 1 to the FARB Mini Column.
   (Centrifuge) Centrifuge at 18,000 x g for 30 sec. Discard the flow -through and return the FARB Mini Column back to the Collection Tube.
   (Vacuum) Apply vacuum at -6 inches Hg until the column have emptied. Switch off the vacuum and release vacuum from the manifold.

9. Add 750 µl of Wash Buffer 2 to the FARB Mini Column.
   (Centrifuge) Centrifuge at 18,000 x g for 30 sec. Discard the flow -through and return the FARB Mini Column back to the Collection Tube.
   (Vacuum) Apply vacuum at -6 inches Hg until the column have emptied. Switch off the vacuum and release vacuum from the manifold.
   -- Note: Make sure that ethanol has been added into Wash Buffer 2 when first use.

10. Repeat step 9 for one more washing.

11. Dry Column:
    Centrifuge at 18,000 x g for 3 min. Discard the flow-through and return the FARB Mini Column back to the Collection Tube.
    -- Important step! This step will prevent subsequent enzymatic reactions from inhibition by the residual wash buffer.

12. Place the FARB Mini Column to a Elution Tube (provided).

13. Add 30~50 µl of RNase-free ddH2O to the membrane center of the FARB Mini Column. Stand the FARB Mini Column at room temperature for 1 min. **-- Important Step!** Ensure that RNase-free ddH2O is dispensed on the membrane center and be absorbed completely. **-- Note! :** Do not use RNase-free water less than suggested volume (<30 µl) to elute RNA. It will lower the RNA yield.

14. Centrifuge the FARB Mini Column at 18,000 x g for 30 sec to elute RNA. Store the RNA at -70°C.

Protocol: Animal Tissues

Please Read Important Notes Before Starting Following Steps.

Additional equipment: • liquid nitrogen & mortar
• a rotor-stator homogenizer, or a 20-G needle syringe.
• ß-Mercaptoethanol
• 70% RNase-free ethanol

A-1. Weight up to 30 mg of tissue sample. Grind the sample in liquid nitrogen to a fine powder with a mortar and transfer the powder to a new microcentrifuge tube (not provided). **-- Note!** Avoid thawing the sample during weighing and grinding.

A-2. Add 350 µl of FARB Buffer and 3.5 µl of ß-Mercaptoethanol. Homogenize the sample by using a rotor-stator homogenizer or by passing the sample lysate through a 20-G needle syringe 10 times. Incubate the sample at room temperature for 5 min.
-- Important step: In order to release more RNA from the harder samples, it is recommended to homogenize the sample by using suitable homogenize equipment, for example, with a rotot-stator homogenizer.

A-3. Follow the Animal Cells Protocol starting from step 3.

(Alternative)

B-1. Place up to 30 mg of tissue sample to a microcentrifuge tube. Add 350 µl of FARB Buffer and 3.5 µl of ß-Mercaptoethanol. Use a provided micropestle to grind the tissue sample thoroughly.

B-2. Homogenize the sample by passing the ground sample through a 20-G needle syringe 10~20 times. Incubate at room temperature for 5 min. **-- Note!** For the tissue samples having low cell amount and hard to disrupt, it is recommended to proceed A1-A3 step above.

B-3. Follow Animal Cells Protocol starting from step 3.

Protocol: Bacteria

Please Read Important Notes Before Starting Following Steps

Additional requirment: • ß-Mercaptoethanol
• 70% RNase-free ethanol
• 30°C water bath or heating block
• 2 ml screw centrifuge tube
• Lysozyme reaction solution: (10 mg/ ml lysozyme; 20 mM Tris-HCl, pH 8.0; 2 mM EDTA; 1.2% Trition)
• Acid-washed glass beads, 500~700 µm

1. Transfer up to 1×10^9 cells well-grown bacterial culture to a 2 ml screw centrifuge tube. **-- Note!** Make sure the amount of total RNA harvested from sample do not excess the column's binding capacity (100 µg) when estimate the sample size. **-- Note!** Too much sample will make cell lysis incompletely and lead to lower RNA yield and purity. If RNA amount is hard to determine on some species, using ≤5×10^8 cells as the starting sample size.
2. Descend the bacterial cells at 4°C by centrifuging at 18,000 x g for 2 min. Remove all the supernatant.
3. Add 100 µl of lysozyme reaction solution. Pipet up and down to resuspend the cell pellet and incubate the sample at 37°C for 10 min.
4. Add 350 µl of FARB Buffer and 3.5 µl of ß-Mercaptoethanol.
5. Add 250 mg of acid-washed glass beads (500~700 µm) and vortex vigorously for 5 min to disrupt the cells.
6. Centrifuge at 18,000 x g for 2 min to spin down insoluble material. Transfer the supernatant to a microcentrifge tube (not provided) and measure the volume of the supernatant. **-- Note!** Avoid pipetting any debris and pellet in the Collection Tube.
7. Follow Animal Cells Protocol starting from step 5.

Protocol: Yeast

Please Read Important Notes Before Starting Following Steps

Additional requirment: • ß-Mercaptoethanol
• 70% RNase-free ethanol

Enzymatic disruption: • Lyticase or zymolase
• Sorbitol buffer (1 M sorbitol; 100 mM EDTA; 0.1% ß-ME)
• 30°C water bath or heating block

Mechanical disruption: • 2 ml screw centrifuge tube
• Acid-washed glass beads (500 ~ 700 µm)

1. Collect up to 5×10^7 of yeast culture at 4°C by centrifuging at 5,000 x g for 10 min . Remove all the supernatant.

2A. Enzymtic disruption:
2A-1: Resuspend the cell pellet in 600 µl of sorbitol buffer.(not provided). Add 200 U of zymolyase (or lyticase) and incubate at 30°C for 30 min. **--Note!** Prepare sorbitol buffer just before use.
2A-2. Centrifuge at 300 x g for 5 min to pellte the spheroplasts. Remove all the supernatant.
2A-3. Add 350 µl of FARB Buffer and 3.5 µl of ß-Mercaptoethanol to the pellet. Vortex vigorously to disrupt the spheroplasts for 1 min. Incbuate the sample mixture at room temperature for 5 min.

2B. Mechanical disruption:
2B-1. Add 350 µl of FARB Buffer and 3.5 µl of ß-Mercaptoethanol to the pellet and vortex vigorously to resuspend the cells completely.
2B-2. Transfer the sample mixture to a 2 ml screw centrifuge tube. Add 250 mg of acid-washed glass beads (500~700 µm) and vortex vigorously for 15 min to disrupt the cells.

3. Follow Animal Cells Protocol starting from step 5.

Protocol: paraffin-embedded tissue

Please Read Important Notes Before Starting Following Steps.

Additional equipment: • xylene & ethanol (96~100%)
• liquid nitrogen & mortar
• a rotor-stator homogenizer or a 20-G needle syringe
• ß-Mercaptoethanol
• 70% RNase-free ethanol

1. Transfer up to 15 mg paraffin-embedded tissue sample to a micro -centrifuge tube (not provided). **-- Remove the extra paraffin to minimize the size of the sample slice.**
2. Add 0.5 ml xylene, mix well and incubate at room temperature for 10 min.
3. Centrifuge at full speed for 3 min. Remove the supernatant by pipetting.
4. Add 0.25 ml xylene, mix well and incubate at room temperature for 3 min.
5. Centrifuge at full speed for 3 min. Remove the supernatant by pipetting.
6. Repeat step 4 and step 5
7. Add 0.3 ml ethanol (96~100%) to the deparaffined tissue, mix gently by vortexing. Incubate at room temperature for 3 min.
8. Centrifuge at full speed for 3 min. Remove the supernatant by pipetting.
9. Repeat step 7 and step 8.
10. Follow Animal tissue Protocol starting from step 1 for sample disruption then follow Animal Cells protocol starting from step 3.

Protocol: RNA clean up

Please Read Important Notes Before Starting Following Steps.

Additional equipment: • ethanol (96~100%)

1. Trandfer 100 µl of RNA sample to a microcentrifuge tube (not provided). -- If the RNA sample is less than 100 µl, add RNase-free water to make the sample volume to 100 µl.
2. Add 300 µl of FARB Buffer and 300 µl of RNase-free ethanol (96~100 %) and mix well by vortexing.
3. Place a FARB Mini Column to a Collection Tube and transfer the ethanol added sample mixture to the FARB Mini Column. Centrifuge at 18,000 x g for 1 min. Discard the flow-through and return the FARB Mini Column back to the Collection Tube.
4. Follow Animal Cells Protocol starting from step 8.

Troubleshooting

- **Low yield**
  - Sample not store well or thaw repeatly
    - Store samples at – 80°C for long-term storage. Frozen samples do not be thawed more than once.
  - RNA Degradation
    - Harvested samples not immediately stabilized.
  - Insufficient mixing with FARB Buffer
    - Mix the sample mixture by plus-vortexing
  - Improper RNA binding condition
    - No ethanol added to the lysate (step 5) or incorrect percentage of ethanol be used.
  - Incorrect RNA elution
    - Ensure that RNase free water was added at the center of the FARB column membrane and absorbed by the membrane .
  - Incorrect preparation of Wash Buffer 2
    - Ensure that the correct volume of ethanol (96~100 %) was added to Wash Buffer 2 when first use.
- **Eluted RNA does not perform well**
  - Residual ethanol contamination
    - Ensure that FARB Column has done centrifugation for an additional 3 min at speed 18,000 x g (step 11) after washing step.

Product category of Favorgen:

For more information please visit Favorgen web site *www.favorgen.com*

Nucleic Acid Extraction - spin column (silica membrane)
- Viral DNA/ RNA Kit
- Viral Nucleic Acid Extraction Kit II
- Circulating Nuleic Acid Isolation Kit

RNA Extraction - spin column (silica membrane)
- Blood/Cultured Cell Total RNA ***Mini/Maxi*** Kit
- Soil RNA Isolation Mini Kit
- Tissue Total RNA ***Mini/ Maxi*** Kit
- Plant Total RNA ***Mini/ Maxi*** Kit
- After Tri-Reagent RNA Clean-Up Kit

96-Well high throughput DNA/ RNA extraction (silica membrane)
- 96-well Gel/ PCR purification kit
- 96-well PCR Clean-Up Kit
- 96-Well Total RNA Kit
- 96 well Viral DNA/RNA extraction kit
- 96-Well Genomic DNA Extraction Kit
- 96-Well Plasmid Kit

DNA Clean-Up - spin column (silica membrane)
- PCR Clean-UP Kit/ • GEL Purification Kit
- GEL/PCR Purification Kit
- ***MicroElute*** GEL/PCR Purification Kit

DNA Extraction - spin column (silica membrane)
- Blood / Cultured Cell Genomic DNA Extraction ***Mini /Midi/Maxi*** Kit
- Plant Genomic DNA Extraction ***Mini/Maxi*** Kit
- Food DNA Extraction Kit
- Milk Bacterial DNA Extraction Kit
- Tissue Genomic DNA Extraction Mini Kit
- FFPE Tissue DNA Extraction ***MicroElute*** Kit
- Fungi/ Yeast Genomic DNA Extraction Mini Kit
- Soil DNA Isolation Mini Kit
- Stool DNA Isolation Mini Kit

Extraction Reagent
- Tri-RNA Reagent (Acid Guanidinium Thiocyanate-Phenol-Chloroform Extraction)

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