FavorPrep™ Tissue Total RNA Mini Kit

For Research Use Only

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

Kit Contents:

Cat. No:	FATRK 000-Mini (4 preps_sample)	FATRK 001 (50 preps)
FARB Buffer	3 ml	25 ml
Wash Buffer 1	3 ml	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
User Manual	1	1

■, 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 x 2
RNase-free Water	6 ml	8 ml x 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
User Manual	1	1

■, Adding Ethanol to the concentrate Wash Buffer 2. ***see Working Buffer Preparation***.

Description:

FavorPrep™ Tissue total RNA Mini Kit is an excellent tool for the purification of high-quality total RNA following the lysis of tissues. It was specially designed for RNA isolation from animal cells, animal tissues, bacteria, yeast, paraffin fixed sample, fungi, and for the subsequent RNA clean-up.

Procedure overview:

The extraction method is silica-based with a chaotropic salt technology presence. The procedure involves lysis of tissues for optimization of a binding condition that promotes the efficient penetration of the nucleic acid into the silica membrane. When compared to the phenol/chloroform and clean-up procedure, this offers better RNA purity and yield, it is much faster and more efficient.

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.

Specifications:

Format/ Principle: spin column/ silica membrane/ chaotropic salt
Operation time: 30 ~ 60 minutes
Length of nucleic acid recovery: > 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 x 10^6)	NIH/3T3 HeLa COS-7 LMH	1 x 10^6 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 x 10^9 cells	60 40
Yeast (up to 5 x 10^7)	S. cerevisiae	1 x 10^7 cells	25

Important Notes:

Notes for Buffers:

1. Make sure that the working environment is RNase-free.
2. Buffers provided in this kit contain irritants, wear gloves, eye protection, 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 reach ~18,000 x g.
5. The vacuum source should reach - 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 fits into the manifold adaptor, and the vacuum pressure is capable of reaching - 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 Protocols:

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

For centrifuge processing:

- A micro-centrifugator capable of reaching ~18,000 X g, with a rotor for1.5 or 2.0 ml micro-centrifuge tubes.

For vacuum processing:

- A micro-centrifugator capable of reaching ~18,000 X g, with a rotor for 1.5~2.0 ml micro-centrifuge tubes.
- A vacuum manifold containing adaptors for Filter Columns and FARB Column. A vacuum capable of reaching -6 inches Hg.

Working Buffer Preparations:

1. "■" **Preparation of Wash Buffer 2**
Add RNase-free ethanol (96~100%) to the Wash Buffer 2 concentrate as the table below indicates. 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 **Recommended:** 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 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.

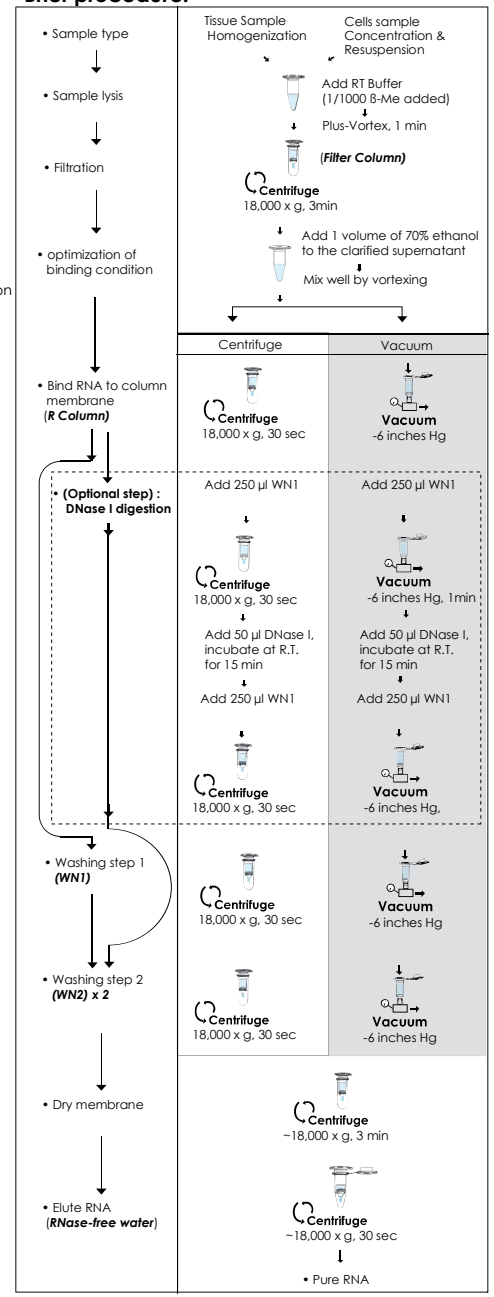
Kit Component: **Wash Buffer 1**

Hazard contents
Guanidine hydrochloride, 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/ face 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 with the 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 incomplete, 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 vortexing, pipette 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 is empty. 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 is empty. 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 is empty. 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 is empty. Switch off the vacuum and release vacuum from the manifold.
   -- Note: Make sure that ethanol has been added into Wash Buffer 2 upon 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 into an 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 onto the membrane center and it is absorbed completely. **-- Note! :** Do not use RNase-free water other than in the suggested volume (< 30 µl) to elute RNA, doing so 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 with the 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 homogenization equipment, e.g. a rotor-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 to A1-A3 steps above.

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

Protocol: Bacteria

Please read Important Notes before starting with the 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: (10mg/ ml lysozyme; 20mM Tris-HCl, pH 8.0; 2mM EDTA; 1.2% Trition)
• Acid-washed glass beads, 500 ~ 700 µm

1. Transfer up to 1x10^9 cells of well-grown bacterial culture to a 2 ml screw centrifuge tube. **-- Note!** Make sure the amount of total RNA harvested from samples does not exceed the column's binding capacity (100 µg) when estimating the sample size. **-- Note!** Too much sample will make cell lysis incomplete and lead to lower RNA yield and purity. If RNA amount is hard to determine on some species, use ≤ 5 x 10^8 cells as the starting sample size.
2. Descend the bacterial cells to 4 °C by centrifuging at 18,000 x g for 2 min. Remove all the supernatant.
3. Add 100 µl of lysozyme reaction solution. Pipette 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 with the 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 x 10^7 of yeast culture at 4 °C by centrifuging at 5,000 x g for 10 min. Remove all the supernatant.

2A. Enzymatic disruption:

2A-1: Resuspend the cell pellet in 600 µl of sorbitol buffer (not provided). Add 200 U of zymolase (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 pellet 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 with the 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 of 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 with the following steps.

Additional equipment: • Ethanol (96~100%)

1. Transfer 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 reach 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 is not stored well or is thawn repeatedly
    - □ Store samples at – 80 °C for long-term storage. Frozen samples should not be thawed more than once.
  - RNA Degradation
    - □ Harvested samples are 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 was 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 upon first use.
- **Eluted RNA does not perform well**
  - Residual ethanol contamination
    - □ Ensure that the FARB Column was centrifuged for an additional 3 min at a speed of 18,000 x g (step 11) after washing

FAVORGEN products:

For more information please visit FAVORGEN's website

Nucleic Acid Extraction - spin column (silica membrane)
- Viral DNA/ RNA Kit
- Viral Nucleic Acid Extraction Kit II
- Viral RNA/ DNA Vacuum Kit
- 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 Kitsin)

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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