FavorPrep™ Tissue Total RNA MicroElute Kit

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

For Research Use Only

Kit Contents:

Cat. No. / preps	FATRM000B (4 preps)	FATRM001B (50 preps)	FATRM001-1B (100 preps)
MR Lysis Buffer	1.5 ml × 2	25 ml	45 ml
Wash R1 Buffer	3 ml	30 ml	60 ml
Wash R2 Buffer ■ (Concentrate)	1.5 ml	15 ml	35 ml
RNase-free ddH_2O	0.5 ml	6 ml	6 ml
Filter Column	4 pcs	50 pcs	100 pcs
RNA Micro Column (Blister packaging)	4 pcs	10 pcs × 5	10 pcs ×10
Collection Tube	4 pcs	50 pcs	100 pcs
Elution Tube	4 pcs	50 pcs	100 pcs
User manual	1	1	1

※ Store the RNA Micro Column to 4~8°C upon receipt

Add RNase-free ethanol (96~100%) to Wash R2 Buffer when first open.

	FATRM000B	FATRM001B	FATRM001-1B
■ Ethanol volume for Wash R2 Buffer	6 ml	60 ml	140 ml

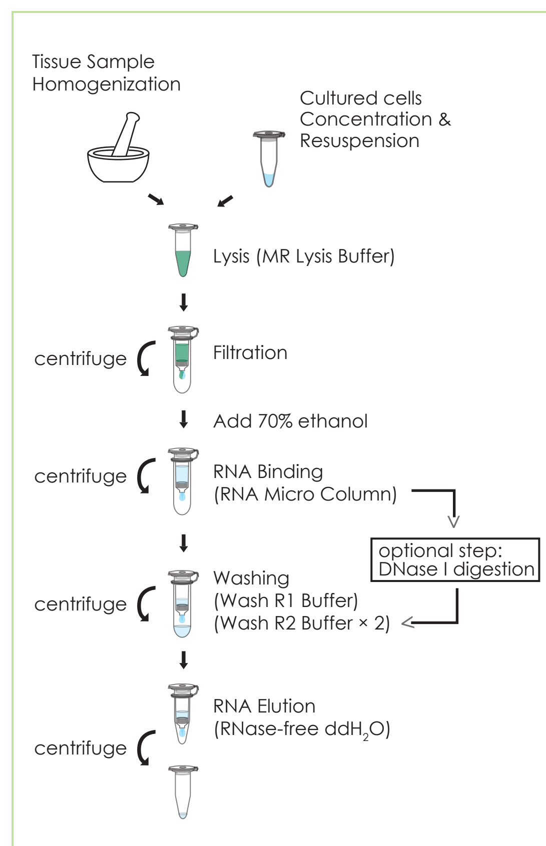
Specification:

Principle: micro spin column (silica matrix)
Operation time: 30~60 mins
Binding capacity: ≤35 μg total RNA/column
Column applicability: centrifugation and vacuum
Minimum elution volume: 10 μl

Sample amount

Sample	Recommended amount of sample used	
Animal cells	NIH/3T3 HeLa COS-7 LMH	≤1×10⁵ cells
Animal Tissue	Embryo Heart Brain Kidney Liver Spleen Lung Thymus	≤5 mg

Brief procedure:



Important Notes:

1. Make sure everything is RNase-free when handling RNA.
2. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
3. **Caution: ß-mercaptoethanol (ß-Me) is hazardous to human health. perform the procedures involving ß-Me in a chemical fume hood.**
4. Add required volume of RNase-free ethanol (96~100%) to Wash R1 Buffer and Wash R2 Buffer when first use.
5. All centrifuge steps are done at full speed (~18,000 xg) in a microcentrifuge.
6. Prepare RNase-free DNase I reaction buffer (1 M NaCl, 10 mM $MnCl_2$, 20 mM Tris-HCl, pH 7.0 at 25°C) and make the final concentration of DNase I to 0.25 U/μl.

General Protocol: Animal Cells

Please Read Important Notes Before Starting Following Steps.

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

1. Collect ≤1×10⁶ cells by centrifuge at 300 xg for 5 mins 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 MR Lysis 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 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 full speed (~18,000 xg) for 2 mins.
4. Transfer ≤300 μl of the clarified supernatant from the Collection Tube to a new microcentrifuge tube. (not provided)
   -Note: Avoid to pipet any turbid debris floating above the pellet when transferring the supernatant.
5. Add 1 volume of RNase-free 70% ethanol and mix well by vortexing.
6. Place a RNA Micro Column to a Collection Tube and transfer the ethanol added sample mixture (including any precipitate) to the RNA Micro Column. Centrifuge at full speed for 1 min, discard the flow-through and return the RNA Micro Column back to the Collection Tube.
7. Optional step: DNase I digestion To eliminate genomic DNA contamination, follow the steps from 7a. Otherwise, proceed to step 8 directly.

   7a. Add 250 μl of Wash R1 Buffer to the RNA Micro Column, centrifuge at full speed for 1 min. Discard the flow-through and return the RNA Micro Column back to the Collection Tube.
   7b. Add 650 μl of RNase-free 70% ethanol to the RNA Micro Column, centrifuge at full speed for 1 min. Discard the flow-through and return the RNA Micro Column back to the Collection Tube.
   7c. Add 30 μl of RNase-free DNase I solution (0.25 U/μl, not provided) to the membrane center of the RNA Micro Column. Place the column on the benchtop for 15 mins.
   7d. Add 250 μl of Wash R1 Buffer to the RNA Micro Column, centrifuge at full speed for 1 min. Discard the flow-through and return the RNA Micro Column back to the Collection Tube.
   7e. After DNase I treatment, proceed to step 9.

8. Add 500 μl of Wash R1 Buffer to the RNA Micro Column, centrifuge at full speed for 1 min. Discard the flow-through and return the RNA Micro Column back to the Collection Tube.
9. Add 650 μl of Wash R2 Buffer to the RNA Micro Column, centrifuge at full speed for 1 min. Discard the flow-through and return the RNA Micro Column back to the Collection Tube.
   -Note: Make sure that ethanol has been added into Wash R2 Buffer when first use.
10. Repeat step 9.
11. Centrifuge the RNA Micro Column at full speed for an additional 3 mins to dry the RNA Micro Column.
    -Important Step! This step will avoid the residual liquid to inhibit subsequent enzymatic reaction.
12. Place the RNA Micro Column to an Elution Tube (provided, 1.5 ml microcentrifuge tube).
13. Add ≥10 μl RNase-free ddH_2O to the membrane center of the RNA Micro Column. Stand the RNA Micro Column for 1 min.
    -Note: The average eluate volume is 10 μl from 12 μl elution volume.
    -Important Step! For effective elution, make sure that RNase-free ddH_2O is dispensed on the membrane center and is absorbed completely.
14. Centrifuge the RNA Micro Column at full speed for 1 min to elute RNA.
15. Store RNA at -70°C.

Protocol: Animal Tissues

Please Read Important Notes Before Starting Following Steps.

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

A-1 Weight up to 5 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 MR Lysis 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 at room temperature for 5 mins.
-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 rotor-stator homogenizer.
A-3. Follow the Animal Cells Protocol starting from step 3.

Protocol: paraffin-embedded tissue

Please Read Important Notes Before Starting Following Steps.

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

1. Transfer ≤5 mg paraffin-embedded tissue sample to a microcentrifuge 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 mins.
3. Centrifuge at full speed for 3 mins. Remove the supernatant by pipetting.
4. Add 0.25 ml xylene, mix well and incubate at room temperature for 3 mins.
5. Centrifuge at full speed for 3 mins. 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 mins.
8. Centrifuge at full speed for 3 mins. 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 MR Lysis Buffer and 250 μl of RNase-free ethanol (96~100%) and mix well by vortexing.
3. Place a RNA Micro Column to a Collection Tube and transfer the ethanol added sample mixture to the RNA Micro Column. Centrifuge at full speed for 1 min and discard the flow-through and return the RNA Micro Column back to the Collection Tube.
4. Follow Animal Cells Protocol starting from step 8.

Protocol: Bacteria

Please Read Important Notes Before Starting Following Steps.

Additional requirement: ß-Mercaptoethanol
RNase-free 70% 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% Triton
Acid-washed glass beads, 500~700 μm

1. Transfer ≤1×10^8 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 (35 μg) when estimate the sample size. 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 ≤ 5×10^7 cells as the starting sample size.
2. Descend the bacterial cells by centrifuge at full speed (~18,000 xg) for 2 mins at 4°C. Remove all the supernatant.
3. Add 100 μl of lysozyme reaction solution. Pipet up and down to resuspend the cell pellet and incubate at 37°C for 10 mins.
4. Add 350 μl of MR Lysis Buffer and 3.5 μl of ß-Mercaptoethanol.
5. Add 250 mg of acid-washed glass beads (500~700 nm) and vortex vigorously for 5 mins to disrupt the cells.
6. Centrifuge at full speed (~18,000 xg) for 2 mins to spin down insoluble material. Transfer the supernatant to a microcentrifge tube (not provided) and measure the volume of the clear lysate.
   -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 requirement: ß-Mercaptoethanol
RNase-free 70% ethanol

Enzymatic disruption: Lyticase or zymolyase
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 ≤ 5×10^6 of yeast culture by centrifuge at 5,000 xg for 10 mins at 4°C. Remove all the supernatant.

2A. Enzymatic disruption:
2A-1: Resuspend the cell pellet in 600 μl sorbitol buffer (1 M sorbitol; 100 mM EDTA; 0.1% ß-ME) (not provided). Add 200 U zymolase or lyticase and incubate at 30°C for 30 mins.
-Note! Prepare sorbitol buffer just before use.
2A-2. Centrifuge at 300 xg for 5 mins to pellet the spheroplasts. Remove all the supernatant.
2A-3. Add 350 μl of MR Lysis Buffer and 3.5 μl of ß-Mercaptoethanol to the pellet. Vortex vigorously to disrupt the spheroplasts for 1 min. Incubate sample mixture at room temperature for 5 mins.
2B. Mechanical disruption:
2B-1. Add 350 μl of MR Lysis 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 and add 250 mg of acid-washed glass beads (500~700 nm) and vortex vigorously for 15 mins to disrupt the cells.

3. Follow Animal Cells Protocol starting from step 5.