FavorPrep™
Tissue Total RNA Mini Kit

User Manual

Cat. No.: FATRK 001 (50 Preps)
FATRK 001-1 (100 Preps)

For Research Use Only

Introduction

The Tissue Total RNA Extraction Kit is specially designed for purification of total RNA from a variety of animcal tissues or cells. The provided micropestle (Mini Format) efficietntly homogenizes tissue samples in a microcentrifuge tube. The method uses detergents and a chaotropic salt to lyse cell and inactivate RNase, than RNA in chaotropic salt is bonded to glass fiber matrix of column. After washing off the contaminants, the purified RNA is eluted by RNase-free water. The entire procedure can be completed in 30 minutes and the purified. ssRNA and dsRNA of >200bps to 1000's of bps in length are efficiently purifed.
The purified RNA is ready for RT-PR, Northern Blotting, Primer Extension and cDNA library construction.

Quality Control

The quality of TissueTotal RNA Mini Kit is tested on a lot-to-lot basis. The kits are tested by isolation of total RNA from 10 mg of mouse liver. More than 20 μg of total RNA was quantified with a spectrophotometer and checked by formaldehyde agarose gel analysis. Finally, RT-PCR was used to ensure the quality of total RNA.

Sample Source: Fresh/Frozen Animal Tissue,
Cultured Animal Cells, Bacterial
Sample Size: Up to 30 mg of tissue
Binding Capacity of Spin Filter: 60μg
Yield: 5~30μg RNA
Formate: Spin column
Operation time: 30 min

Procedure

	FATRK001 (50 preps)	FATRK001-1 (100preps)
FARB Buffer	30 ml	60 ml
Wash Buffer 1	30 ml	50 ml
Wash Buffer 2 (conc.)*	15 ml	35 ml
RNase-free Water	6 ml	6 ml
FARB column	50pcs	100pcs
Filter Column	50pcs	100pcs
Micropestles (RNase free)	50pcs	100pcs
Elution Tube	50pcs	100pcs
Collection Tube	100pcs	200pcs

- β-Meraptoethanol (β-ME) must be added to FARB Buffer before use.
- Add 4 times volume of ethanol (96%~100%) to Wash Buffer before first use (50/100 ml).

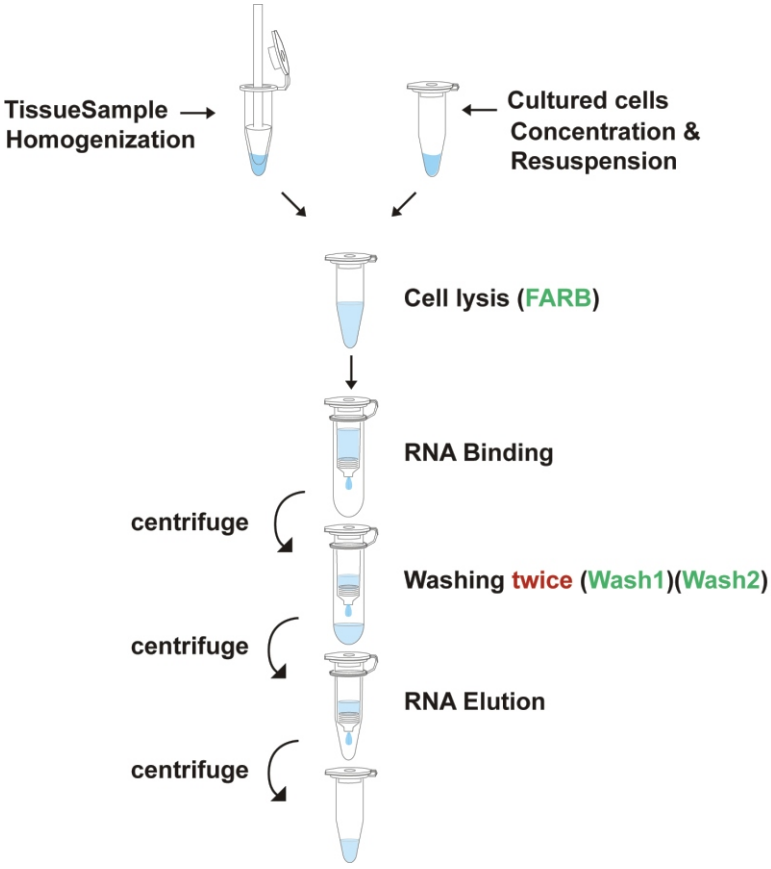
Caution

The component contains irritant agent. During operation, always wear a lab coat, disposable gloves, and protective goggles.

References

(1) Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615.

Prcedure


TissueSample
Homogenization
Cultured cells
Concentration &
Resuspension
Cell lysis (FARB)
RNA Binding
centrifuge
Washing twice (Wash1)(Wash2)
centrifuge
RNA Elution
centrifuge

Important Notes

1. Make sure everything is RNase-free when handing RNA.

2. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.

3. Pipet a required volumn of FARB Buffer to another RNase-free container and add 10 µl β-mercaptoethanol (β-ME) per 1 ml FARB Buffer before use.

4. Add 6 ml ethanol (96~100%) to Wash Buffer 2 when first open.

5. All centrifuge steps are done at full speed (14,000 rpm or 10,000 x g)in a microcentrifuge.

6. Dilute RNase-free DNase 1 in dilution buffer (150 mM NaCl, 1 mM $MgCl_2$, 10 mM Tris-HCl, pH 7.5) to final conc. = 0.5 U/µl.

7. Some genomic DNA will alse be copurified with RNA. The oplional DNase treatment is threrfore required when DNA-free RNA is desired.

Sample amount and yield

Sample	Recommended amount of sample used		Yield (µg)
Animal cells (up to 5 X 10^6)	NIH/3T3	1 x 10^6 cells	10
	HeLa	1 x 10^6 cells	15
	COS-7	1 x 10^6 cells	30
	LMH	1 x 10^6 cells	12
Animal tissues (Mouse/rat) (up to 30 mg)	Embryo	10 mg	25
	Heart	10 mg	10
	Brain	10 mg	10
	Kidney	10 mg	30
	Liver	10 mg	50
	Spleen	10 mg	35
	Lung	10 mg	15
	Thymus	10 mg	45
Bacteria	E. Coli	1 x 10^9 cells	60
	B. Subtilis	1 x 10^9 cells	40
Yeast (up to 5 x 10^7)	S. Cerevisia	1 x 10^7 cells	20

Protocol

***Please Read *Important Notes* Before Starting The Following Steps.**

1. For Animal Cells

1. Pellet 1 - 5 x 10^6 cells by centrifuging (300 x g) for 5 min. Remove all supernatant.

2. Add 350 µl of FARB Buffer (β-ME added) to the cell pellet and vortex vigorously.

3. Place a Filter Column into a Collection Tube. Transfer the sample mixture to Filter Column and centrifuge at full speed (14,000 rpm or 10,000 x g) for a min.

4. Transfer the clarified supernatant from the Collection Tube to a new microcentrifuge tube (not provided) and adjust the volume of the clear lysate.
   - Avoid pipetting any debris and pellet in the Collection Tube.

5. Add 1 volume of 70 % ethanol to the clear lysate and mix well.

6. Place a FARB Mini Column into a Collection Tube. Transfer the sample mixture (ethanol added)(including any precipitate) to FARB Mini Column. Centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min and discard the flow-through.

7. (Optional): To eliminate genomic DNA contamination, follow the steps from 7a. Otherwise, proceed to step 8 directly.
   • This step is required when DNA-free RNA is desired.

   7a. Add 250 µl of Wash Buffer 1 to wash FARB Mini Column. Centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min then discard the flow-through.

   7b. Add 100 µl of RNase-free Dnase 1 solution (2U/µl, not provided) to the membrane center of FARB Mini Column. Place the Column on the benchtop for 15 min.

   7c. Add 250 µl of Wash Buffer 1 to wash FARB Mini Column. Centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min then discard the flow-through.

   7d. After DNase 1 treatment, proceed to step 9.

8. Add 500 µl of Wash Buffer 1 to wash FARB Mini Column. Centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min then discard the flow-through.

9. Wash FARB Mini Column twice with 700 µl of Wash Buffer 2 by centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min then discard the flow-through.
   • Make sure that ethanol has been added into Wash Buffer 2 when first open.

10. Centrifuge at full speed (14,000 rpm or 10,000 x g) for an additional 3 min to dry the column.
    - **Important Step!** This step will avoid the residual liquid to inhibit the subsequent enzymatic reactions.

11. Place FARB Mini Column to a Elution Tube.

12. Add 50 µl of RNase-free Water to the membrane center of FARB Mini Column. Stand FARB Mini Column for 1 min.
    - **Important Step!** For effective elution, make sure that RNase-free Water is dispensed on the membrane center and is absorbed completely.

13. Centrifuge at full speed (14,000 rpm or 10,000 x g) for 2 min to elute RNA.

14. Store RNA at -70°C.

2. For Animal Tissue

1. (For fresh sample and frozen sample) Cut up to 30 mg tissue sample to a microcentrifuge tube (not provided). Or you can grind the tissue sample in liquid nitrogen then transfer the powder to a new microcentrifuge tube.

2. Add 350 µl of FARB Buffer (β-ME added) to the sample and use provided Micropestle to grind the tissue sample completely.
    - Grind the sample a few times to make it break more completely.

3. Follow the Animal Cells Protocol starting from setp 3.

3. For Bacteria

1. Transfer 1 ml well-grown bacterial culture (or up to 1 x 10^9 cells) to a microcentrifuge tube (not provided).

2. Descend the bacterial cells by centrifuge at full speed (14,000 rpm or 10,000 x g) for 2 min and discard the supernatant completely.

3. Resuspend the cell pellet in 100 µl RNase-free lysozyme reaction solution (not provided)(20 mg/ml lysozyme; 20 mM Tris-HCl, pH 8.0; 2 mM EDTA; 1.2 % Trition).

4. Incubate at 37°C for 10 min.

5. Add 350 µl of FARB Buffer (β-ME added) to the sample and mix well by vortexing.

6. Centrifuge at full speed (14,000 rpm or 10,000 x g) for 2 min to spin down insoluble materials and transfer the supernatant to a microcentrifuge tube (not provided).

7. Add 250 µl of ethanol (96 - 100 %) to the clear lysate and mix by pietting.

8. Follow the Animal Cells Protocol starting from step 6.

4. For Yeast

1. Transfer 3 ml log-phase (OD600 = 10) yeast culture to a microcentrifuge tube (not provided).

2. Descend the yeast cells by centrifuging at 7,500 rpm (5,000 x g) for 10 min and discard the supernatant completely.

3. Resuspend the cell pellet in 600 µl sorbitol buffer (not provided)(1 M sorbitol; 100 mM EDTA; 0.1 % β-ME). Add 200 U zymolase or lyticase and incubate at 30°C for 30 min.
   • prepare sorbitol buffer just before use.

4. Centrifuge at 7,500 rpm (5,000 x g) for 5 min. Remove the supernatant by pipetting.

5. Add 350 µl of FARB Buffer (β-ME added) to the sample and mix well by vortexing.

6. Centrifuge at full speed (14,000 rpm or 10,000 x g) for 2 min to spin down insoluble materials and transfer the supernatant to a microcentrifuge tube (not provided).

7. Follow the Animal Cells Protocol starting from step 5