FavorPrep™
Tissue Total RNA Maxi Kit (Sample)

User Manual

For Research Use Only

Cat. No.: FATRK 000-Maxi
(sample: 2 Preps)

v.1005

Favorgen Biotech Corporation

Handguarters

Ping-Tung Agricultural Biotechnology Park
No. 37, Nong-Ke Rd., Ping-Tung 908,
Taiwan
■ Tel: +886-8-762-1829
■ Fax: +886-8-762-0971
Email: service@favorgen.com
Web Site: www.forgen.com

Introduction

FavorPrep Tissue Total RNA Extraction Maxi Kit is desigened for extraction of total RNA from a variety of animal tissues and cells. Some specially modified protocols are developed for other samples, such as bacteria and yeast. This method first lyses cells by using a chaotropic salt , then binds RNA to silica-based membranes, washes RNA with ethanol-contained wash buffer and then elutes purified RNA by RNase-free ddH_2O. It takes 60 min for an entire procedure, and the purified RNA is ready for RT-PCR, northern blotting, primer extension and cDNA library contruction.

Sample amount:

0.5 ~1 g of animal tissue

Up to 5 X 10^{10} bacteria cells

Up to 5 X 10^{9} yeast culture

Up to 5 ×10^{8} of animal cells

Handling time: about 60 min

Kit Contents

Cat. No. / preps	FATRK000-Maxi (2 preps)
FARB Buffer	30 ml
Wash Buffer 1	30 ml
Wash Buffer 2 (concentrated)	12 ml *
RNase-free ddH_2O	5 ml
Filter Column	2 pcs
FARB Mini Column	2 pcs
Elution Tube (15 ml tube)	2 pcs
User manual	1

* Add 48 ml ethanol (96-100 %) to each Wash Buffer 2 when first open.

Special Protocol: (For Yeast)

1. Transfer up to 5 X 10^{9} ml of log-phase (OD600=10) yeast culture to a 50 ml centrifuge tube. (not provided)
2. Descend the yeast cells by centrifug at 500 x g at 4 °C for 5 min and discard the supernatant completely.
3. Resuspend the cell pellet in 2.5 ml of enzymatic lysis buffer (20 mg/ml lyticase or zymolase; 1M sorbitol; 100mM EDTA; 0.1% ß-ME) (not provided).
   And incubate at 30 °C for 30 min.
   --Prepare sorbitol buffer just before use.
4. Centrifuge at 500 x g at room temperature for 5 min to pellet spheroplasts and discard the supernatant completely.
5. Add 14 ml of FARB Buffer (ß-ME added) to the sample and mix well by vortexing. Incubate at room temperature for 5 minutes.
6. Centrifuge at full speed for 5 min to spin down insoluble materials and transfer the clarified supernatant to a 50 ml tube (not provided).
7. Add an equal volume of 70% ethanol to the clear lysate and mix by pipetting.
8. Follow the Animal Tissue Protocol starting from step 7.

Special Protocol: (For Animal Cells)

1. Pellet Up to 5 ×10^8 of animal cells by centrifuge at 300 x g for 5 min. Discard the supernatant completely.
2. Add 14 ml of FARB Buffer (ß-ME added) to the cell pellet and vortex vigorously. Incubate at room temperature for 5 min.
   (For preparation of FARB Buffer (ß-ME added), see Important Note: 3)
3. Place a Filter Maxi Column in a 50 ml tube (not provided), and transfer the sample mixture to Filter Maxi Column, centrifuge at full speed for 5 min.
4. Transfer the clarified supernatant from previous step to a clean 50 ml tube (not provided) and adjust the volume of the clear lysate.
   --Avoid pipetting any debris and pellet from this Collection Tube.
5. Add an equal volume of 70% ethanol to the clear lysate and mix well by pipetting.
6. Follow the General Protocol starting from step 7.

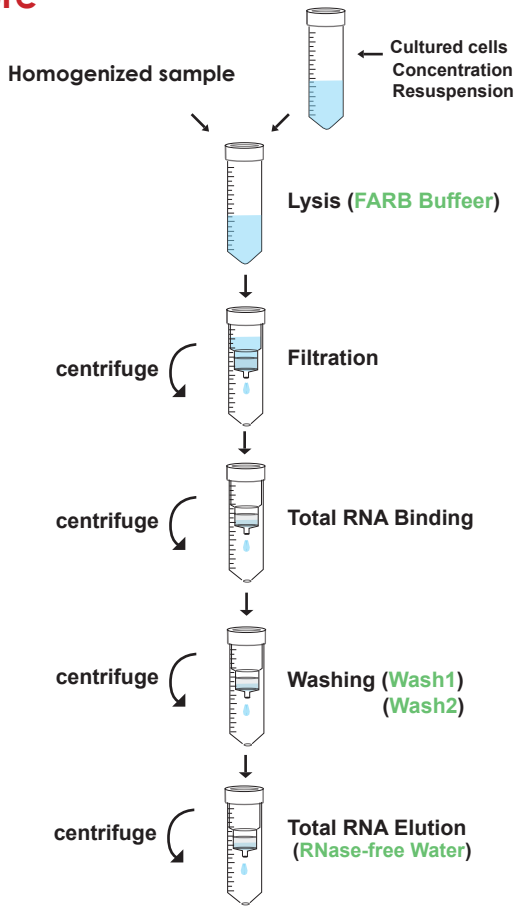
Special Protocol: (For Bacteria)

1. Transfer Up to 5 X 10^{10} of well-grown bacterial to a entrifuge tube (not provided).
2. Descend the bacterial cells by centrifuge at >3,000 x g for 5 min and discard the supernatant completely.
3. Resuspend the cell pellet in 1 ml of RNase-free lysozyme reaction solution (20mg/ml lysozyme; 20mM Tris-HCl, pH 8.0; 2mM EDTA; 1.2% Trition) (not provided).
4. Incubate at 37°C for 10 min.
5. Add 14 ml of FARB Buffer (ß-ME added) to the sample and mix well by vortex. Incubate at room temperature for 5 min.
   (For preparation of FARB Buffer (ß-ME added), see Important Note: 3)
6. Centrifuge at full speed for 5 min to spin down insouble material and transfer the supernatant to a 50 ml tube. (not provided)
7. Add an equal volume of 70% ethanol to the clear lysate and mix by pipetting.
8. Follow the General Protocol starting from step 7.

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 hese buffers.
3. Pipet a required volume of FARB Buffer to another RNase-free container and add 10 µl ß-mercaptoethanol (ß-ME) per 1ml FARB Buffer before use.
4. Add required volume of RNase-free ethanol (96~100%) to Wash Buffer 2 as bottle indicated when first open.
5. Dilute RNase-free DNase 1 in reaction buffer (150mM NaCl, 1 mM $MgCl_2$, 10 mM Tris HCl, pH 7.5) to final conc. = 2KU/ml. (1 ml /preparation)
6. Use a centrifuge with a swinging bucket rotor for 15ml (Midi) or 50ml (Maxi) in all centrifugation steps. The maximum speed should be 3500-5000 rpm or 3000-5000 x g.

Brief Procedure



Genernal Protocol: (For Animal Tissue)

Please Read Important Notes Before Starting The Following Steps.

Additional equipment: a 20-G needle syringe

1. **Cut off 0.5 g (up to 1 g) of tissue sample and grind the tissue sample completely under liquid nitrogen to a find powder then transfer the powder to a 50 ml centrifuge tube.**

   Note: Do not use to much sample in this RNA extraction procedure ! It is important to use the correct number of starting cells in order to obtain optimal RNA yield and purity.

2. **Add 14 ml of FARB Buffer (ß-ME added) to the sample and shear this tissue sample by passing lysate through a 20-G needle syringe 10 times**

   Note: In order to release all the RNA in the sample, it is required to disrupt the sample completely. Different samples require different methods (ex: disruptor equipment) to achieve complete disruption.

3. **Incubate the sample mixture at room temperture for 5 minutes.**

4. **Place a Filter Maxi Column in a clean 50 ml tube (not provided), and transfer the sample mixture to Filter Maxi Column, centrifuge at full speed for 5 min.**

5. **Transfer the clarified supernatant from previous step to a clean 50 ml tube (not provided), and adjust the volume of the clear lysate.**
   --Avoid to disrupt any debris and pellet when transfer the supernatant.

6. **Add an equal volume of 70% ethanol to the clear lysate and mix well by vortexing.**

7. **Place a FARB Maxi Column in a clean 50 ml tube (not provided), and transfer 14 ml of the ethanol added sample (including any precipitate) to FARB Maxi Colum. Centrifuge at full speed for 5 min. Discard the flow-through and place the FARB Maxi Column back in 50 ml centrifuge Tube. Then repeat this step for the rest sample mixture.**

8. **(Optional): To eliminate genomic DNA contamination, follow the steps from 8a. Otherwise, proceed to step 9 directly.**

   8a. Add 7 ml of Wash Buffer 1 to wash FARB Maxi Column, centrifuge at full speed for 2 min. Discard the flow-through and place the FARB Maxi Column back in 50 ml centrifuge Tube.

   8b. Add 0.5 ml of RNase-free DNase 1 solution (2U/µl, not provided) to the membrane center of FARB Maxi Column. Place the Column on the benchtop for 10 min.

   8c. Add 7 ml of Wash Buffer 1 to wash FARB Maxi Column, centrifuge at full speed for 2 min. Discard the flow-through and place the FARB Maxi Column back in 50 ml centrifuge Tube.

   8d. After DNase 1 treatment, proceed to step 10.

9. **Add 12.5 ml of Wash Buffer 1 to wash FARB Maxi Column, centrifuge at full speed for 2 min. Discard the flow-through and place the FARB Maxi Column back in 50 ml centrifuge Tube.**

10. **Wash FARB Maxi Column twice with 12.5 ml of Wash Buffer 2 by centrifuging at full speed for 2 min. Discard the flow-through and place the FARB Maxi Column back in 50 ml centrifuge Tube.**
    --Make sure that ethanol has been added into Wash Buffer 2 when first open.

11. **Centrifuge at full speed (> 4,000 x g) for an additional 10 min to dry the FARB Maxi Column.**
    --**Important Step!** This step will avoid the residual liquid to inhibit subsequent enzymatic reaction.

12. **Place FARB Maxi Column in Elution Tube (50 ml tube, provided).**

13. **Add 500~1000 µl of RNase-free Water to the membrane center of FARB Maxi Column. Stand FARB Maxi Column for 5 min.**
    --**Important Step!** For effective elution, make sure that RNase-free Water is dispensed on the membrane center and is absorbed completely.

14. **Centrifuge at full speed for 5 min to elute RNA.**

15. **Store RNA at -70°C.**