FavorPrep™
Blood / Cultured Cell
Total RNA Purification Maxi Kit

User Manual

Cat. No.: FABRK 003 (10 Preps)
FABRK 003-1 (24 Preps)

For Research Use Only

v.1207

Introduction

FavorPrep Blood/ Cultured Cell Total RNA Extraction Maxi Kit is desigened for extraction of total RNA from whole blood and cultured 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 Water. 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:

3~10 ml of human whole blood

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	FABRK003 (10 preps)	FABRK003-1 (24 preps)
10X RL Buffer	100 ml	200 ml
FARB Buffer	150 ml	180 ml x 2
Wash Buffer 1	135 ml	160 ml x 2
Wash Buffer 2 (concentrated)	54 ml	45 ml x 3
RNase-free Water	12 ml	30 ml
Filter Column	10 pcs	24 pcs
FARB Maxi Column	10 pcs	24 pcs
Elution Tube (50 ml tube)	10 pcs	24 pcs
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* Add 216 ml / 180 ml ethanol (96-100 %) to each Wash Buffer 2 when first open.

Special Protocol: (For Yeast)

1. Transfer up to 5 X 10^{9} yeast cells 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 Genernal Protocol starting from step 12.

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 Genernal Protocol starting from step 12.

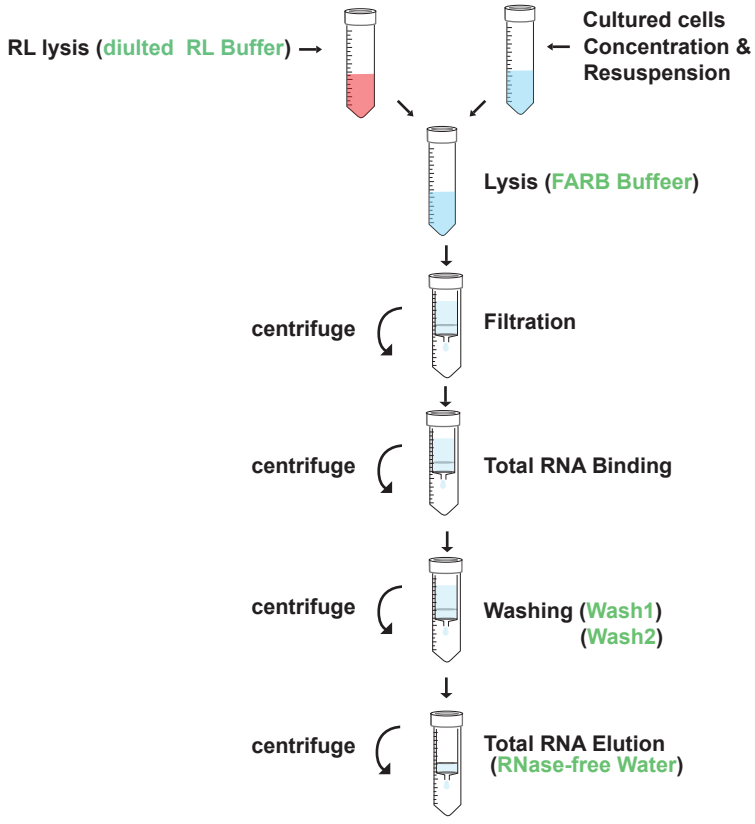
Special Protocol: (For Bacteria)

1. Transfer Up to 5 X 10^{10} of well-grown bacterial to a centrifuge 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 13 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 Genernal Protocol starting from step 12.

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 Human Whole Blood)

Please Read Important Notes Before Starting The Following Steps.

1. **Collect fresh human blood in an anticoagulant-treat collection tube.**

2. **Add 3 ~10 ml of human whole blood to an appropriately sized centrifuge tube (15 ml or 50 ml tube). (not provided)**

3. **Mix 5 volume of diluted RL Buffer with 1 volume of the sample and mix well by inversion.**
   Note: RL buffer is provided as a 10 x concentrate which would must be diluted with sterile deionized water before use.
   For example, add 25 ml of diluted RL Buffer to 5 ml of blood sample.

4. **Incubate at room temperature for 5 min. Vortex briefly 2 times during incubation.**

5. **Centrifuge for 5 min at 500 x g to form a cell pellet and discard the supernatant completely.**

6. **Add 2 volume of diluted RL Buffer to wash the cell pellet by briefly vortexing.**

7. **Centrifuge for 5 min at 500 x g to form a cell pellet again and discard the supernatant completely.**

8. **Add 12.5 ml of FARB Buffer (ß-ME added) to the cell pellet and vortex vigorously. Incubate at room temperature for 3 min to lyse cells completely.**
   (For preparation of FARB Buffer (ß-ME added), See Important Note: 3)

> **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.**

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

10. **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.

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

12. **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.**
    ---The maximum capacity of FARB Midi Column is 14 ml, repeat Step 7 for the remaining sample mixture.

13. **(Optional): To eliminate genomic DNA contamination, follow the steps from 13a. Otherwise, proceed to step14 directly.**

    13a. **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.**

    13b. **Add 1 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.**

    13c. **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.**

    13d. **After DNase 1 treatment, proceed to step 15.**

14. **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.**

15. **Wash FARB Maxi Column twice with 12.5 ml of Wash Buffer 2 by Centrifuge 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.

16. **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.

17. **Place FARB Maxi Column to Elution Tube (50 ml tube, provided).**

18. **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.

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

20. **Store RNA at -70°C.**