FavorPrep™ Blood/ Cultured Cell Total RNA Midi Kit

User Manual

For Research Use Only

Cat. No. : FABRK002 (20 Preps)
FABRK002-1 (50 Preps)

Introduction

FavorPrep Blood/ Cultured Cell Total RNA Extraction Midi Kit is designed 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 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:

5~10 ml of human whole blood

Up to 1 X 10^{10} bacteria cells

3~10 ml of log-phase (OD600=10) yeast culture

3 × 10^7~7 ×10^7 of animal cells

Handling time: about 60 min

Kit Contents

	FABRK002 (20 prsps)	FABRK002-1 (50 prsps)
10 X RL Buffer	185 ml	185 ml X 2
FARB Buffer	110 ml	110 ml X 2
Wash Buffer 1	110 ml	110 ml X 2
Wash Buffer 2 (concentrated)	35 ml *	35 ml ** X 2 +15 ml
RNase-free ddH_2O	20 ml	50 ml
Filter Column	20 pcs	50 pcs
FARB Mini Column	20 pcs	50 pcs
Elution Tube (15 ml tube)	20 pcs	50 pcs
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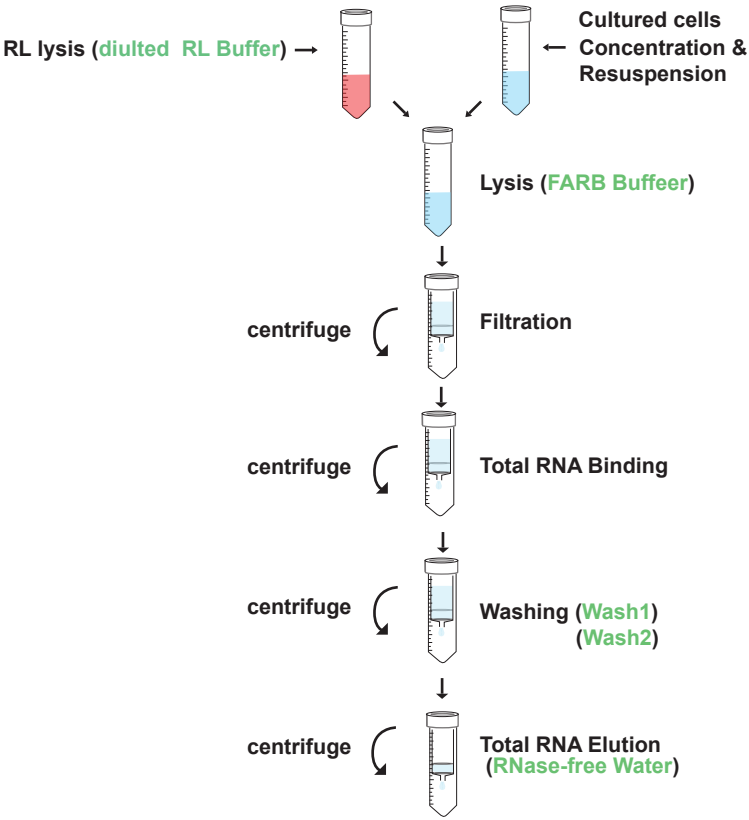
* Add 60 ml ethanol (96-100 %) to Wash Buffer 2 when first open.
** Add 140 ml ethanol (96-100 %) to Wash Buffer 2 when first open.

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 to final conc. = 0.5 U/µl.

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)**
   --If the sample volume is more than 10 ml, use two 50 ml tube as the sample container.

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 1 min at 4,500 rpm 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 1min at 4,500 rpm to form a cell pellet again and discard the supernatant completely.**

8. **Add 4 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)

9. **Place a Filter Mid Column into a clean 15 ml tube (not provided), and transfer the sample mixture to Filter Column, centrifuge at 6,000~8,000 rpm for 5 min.**

10. **Transfer the clarified supernatant from previous step to a clean 15 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 Midi Column into a clean 15 ml tube (not provided), and transfer the ethanol added sample (including any precipitate) to FARB Midi Colum, centrifuge at 6,000 rpm for 5 min and discard the flow-through.**

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

13a. Add 2 ml of Wash Buffer 1 to wash FARB Midi Column. Centrifuge at 6,000 rpm for 2 min then discard the flow-through.

13b. Add 300 µl of RNase-free DNase 1 solution (0.5U/µl, not provided) to the membrane center of FARB Mini Column. Place the Column on the benchtop for 15 min.

13c. Add 2 ml of Wash Buffer 1 to wash FARB Midi Column. Centrifuge at 6,000 rpm for 2 min then discard the flow-through.

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

14. Add 4 ml of Wash Buffer 1 to wash FARB Midi Column. Centrifuge at 6,000 rpm for 2 min then discard the flow-through.

15. Wash FARB Midi Column twice with 4 ml of Wash Buffer 2 by centrifuging at 6,000 rpm for 2 min then discard the flow-through.
--Make sure that ethanol has been added into Wash Buffer 2 when first open.

16. Centrifuge at 6,000~8,000 rpm for an additional 10 min to dry the column.
--**Important Step!** This step will avoid the residual liquid to inhibit subsequent enzymatic reaction.

17. Place FARB Midi Column to Elution Tube (15 ml tube, provided).

18. Add 250~500 µl of RNase-free Water to the membrane center of FARB Midi Column. Stand FARB Midi 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 6,000~8,000 rpm for 5 min to elute RNA.

20. Store RNA at -70ºC.

(For Animal Cells)

1. **Pellet 3 × 10^7~7 ×10^7 cells by centrifuge at 300 x g for 5 min. Remove all the supernatant.**
2. **Add 4 ml of FARB Buffer (ß-ME added) to the cell pellet and vortex vigorously. Incubate at room temperature for 3 min.**
   (For preparation of FARB Buffer (ß-ME added), see Important Note: 3)
3. **Place a Filter Midi Column into a 15 ml tube (not provided), and transfer the sample mixture to Filter Column, centrifuge at 6,000~8,000 rpm for 5 min.**
4. **Transfer the clarified supernatant from previousstep to a clean 15 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 Animal Cells Protocol starting from step 12.**

(For Bacteria)

1. **Transfer up to 1x10^{10} cells of well-grown bacterial to a entrifuge tube (not provided).**
2. **Descend the bacterial cells by centrifuge at 3,000 rpm for 5 min and discard the supernatant completely.**
3. **Resuspend the cell pellet in 500 µl 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 4 ml of FARB Buffer (ß-ME added) to the sample and mix well by vortex. Incubate at room temperature for 3 min.**
   (For preparation of FARB Buffer (ß-ME added), see Important Note: 3)
6. **Centrifuge at 6,000~8,000 rpm for 5 min to spin down insouble material and transfer the supernatant to a 15 ml tube. (not provided)**
7. **Add an equal volume of 70% ethanol to the clear lysate and mix by pipetting.**
8. **Follow the Animal Cells Protocol starting from step 12.**

(For Yeast)

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

2. **Descend the yeast cells by centrifug at 3,000 rpm for 5 min and discard the supernatant completely.**

3. **Resuspend the cell pellet in 1.2 ml of sorbitol buffer (1M sorbitol; 100mM EDTA; 0.1% ß-ME) (not provided). Add 200 U zymolase or lyticase and incubate at 30 °C for 30 min.**
   --Prepare sorbitol buffer just before use.

4. **Centrifuge at 6,000rpm for 5 min. Remove the supernatant by pipetting.**

5. **Add 4 ml of FARB Buffer (ß-ME added) to the sample and mix well by vortexing.**

6. **Centrifuge at 6,000 rpm or for 5 min to spin down insoluble materials and transfer the supernatant to a 15 ml tube (not provided).**

7. **Add an equal volume of 70% ethanol to the clear lysate and mix by pipetting.**

8. **Follow the Animal Cells Protocol starting from step 12.**

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