FavorPrep™
Blood / Cultured Cell
Total RNA Purification Mini Kit

User Manual

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

For Research Use Only

Introduction

The Blood/Cultured Cell Total RNA Purification Mini Kit is designed for purification of total RNA from cultured cells and fresh whole blood using detergents and the chaotropic salt-lysis method to lyse the cells and inactivate RNase without the use of hazardous solvents such as phenol. The lysate is applied to provid Lysate Filter columns (Mini System) to remove cell debris and ensure complete cell lysis. RNA in chaotropic salt solutions binds to the glass fiber matrix of the columns.
Following washing off of contaminants, the purified RNA is eluted by RNase-free water, ssRNA and dsRNA of >200 bps to 1000's of bps in length are efficiently, which is suitable for direct use in RT-PCR, Northern blotting, primer extension and cDNA library construction.
The entire procedure can be completed in 30-60 minutes.

Quality Control

The quality of Blood/Cultured Cell Total RNA Mini Kit is tested on a lot-to-lot basis. The kits are tested by isolation of total RNA from 300 µl of fresh human whole blood. More than 1 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: Animal Tissue, cultured cells, blood and other body fluids
Sample Size: Up to 300 µl of human whole blood, $1x10^9$ bacterial cells, $5x10^6$mammalian cultured cells
Formate: Spin column
Operation time: About 30 min

Kit Contents

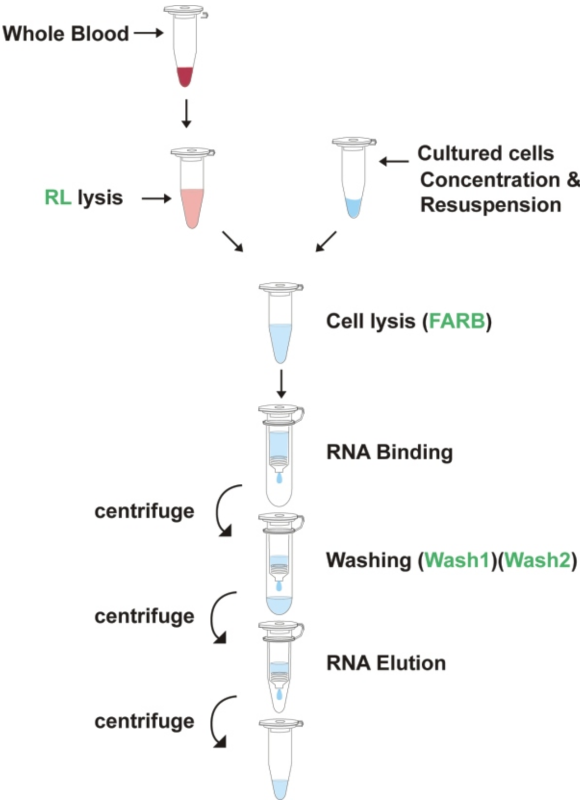
	FABRK001 (50preps)	FABRK001-1 (100preps)
RL Buffer	90 ml	180 ml
FARB Buffer	30 ml	60 ml
Wash Buffer 1	30 ml	60 ml
Wash Buffer 2*	15 ml	35 ml
RNase-free water	6 ml	6 ml
FARB Column	50 pcs	100 pcs
Filter Column	50 pcs	100 pcs
1.5 ml Elution Tube	50 pcs	100 pcs
2 ml Collection Tube	100 pcs	200 pcs

*Add 60 ml / 140 ml ethanol (96~100%) to Wash Buffer 2 before first use.

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 volume of FARB Buffer to another RNase-free container and add 10 μl *β*-mercaptoethanol (*β*-ME) (not provided) per 1 ml FARB Buffer before use.
4. For FABRK001, add 60ml ethanol (96 - 100%) to Wash Buffer 2 and FABRK001-1, add 140ml ethanol (96 - 100%) to Wash Buffer 2 when first open..
5. 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. = 0.5U/μl

Procedure



Protocol:(Purification of total RNA from fresh human blood)

1. Collect fresh human blood in the anticoagulant-treated collection tubes.
2. Add 200~300 μl human whole blood to an appropriately sized microcentrifuge tube (1.5ml or 2.0ml tube). (Not Provided)
   --If the sample volume is more than 200 μl, use a 2.0ml tube as the sample container.
3. Mix 5 volumes of RL Buffer with 1 volume of the sample and mix well by inversion.
   (For example, Mix 1250μl of RL Buffer with 250 μl of the 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 600 μl of RL Buffer to resuspend the cell pellet by briefly votexing.
7. Centrifuge for 1 min at 4,500 rpm to form a cell pellet **again** and discard the supernatant completely.
8. Add 350 μl of FARB Buffer (β-ME added) to the cell pellet and mix well by vortexing vigorously. Incubate at room temperature for 3 min.
   (For Preparation of FARB Buffer (β-ME added), See Important Note:3).
9. Place a Filter Column into a Collection Tube, transfer the mixture to filter column and centrifuge at full speed (14,000 rpm) for 2 min.
10. Ttransfer 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 from this Collection Tube.

11. Add 1 volume of 70% ethanol the the clear lysate and mix well by pipetting.
12. Place a FARB Mini Column into a CollectionTube, And transfer 750 µl of the ethanol-added sample (including any precipitate) to FARB Mini Column. Centrifuge at full speed (14,000 rpm)for 1 min and discard the flow-through.
13. Repeat step 12 for rest of the sample.

Optional Step: DNA residue degradation

14. To eliminate genomic DNA contamination, follow the steps from 14a. Otherwise, proceed to step 15 directly.
14a. Add 250 µl of Wash Buffer 1 to wash FARB Mini Column. Centrifuge at full speed (14,000 rpm) for 1 min then discard the flow-through.
14b. Add 100 µl of RNase-free DNase 1 solution (0.5U/µl, provide by user) to the membrane center of FARB Mini Column. Place the Column on the benchtop for 15 min.
14c. Add 250 µl of Wash Buffer 1 to wash FARB Mini Column. Centrifuge at full speed (14,000 rpm) for 1 min then discard the flow-through.
14d. After DNase 1 treatment, proceed to step 16.

15. Add 500 μl of Wash Buffer 1 to wash FARB Mini Column. Centrifuge at full speed (14,000 rpm) for 1 minute then discard the flow-through.
16. Wash FARB Mini Column twice with 700 μl of Wash Buffer 2 by centrifuge at full speed (14,000 rpm) for 1 minute then discard the flow-through.
    Caution: Make sure that ethanol has been added into Wash Buffer 2 when first open.
17. Centrifuge at full speed (14,000 rpm) for 3 minutes to dry the column matrix.
    Caution: Important step! This step will avoid the residual liquid to inhibit the subsequent enzymatic reactions.
18. Place FARB Mini Column into Elution Tube.
19. Add 50 μl of RNase-free water to the membrane center of FARB Mini Column. Stand FARB Mini Column for 1 min.
    Caution: Important step! For effective elution, make sure that RNase-free water is dispensed on the membrane center and is absorbed completely.
20. Centrifuge at full speed (14,000 rpm) for 2 minute to elute purified RNA.
21. Store RNA at -70℃.

Protocol:(Purification of total RNA from animal Cells)

1. Pellet 1 - 5 x 10^6 cells by centrifuging (300 xg) for 5 min. Remove all supernatant.
2. Add 350 μl of FARB Buffer (β-ME added) to the cell pellet and mix well by vortexing vigorously. Icubate at room temperature for 3 min. (For Preparation of FARB Buffer (β-ME added), See Important Note:3).
3. Place a Filter Column into a CollectionTube, and the sample to Filter Column and centrifuge at full speed (14,000 rpm) for 2 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. **Caution:** Avoid pipette any debris and pellet.
5. Add 1 volume of 70% ethanol to the clear lysate and mix well.
6. Place a FARB Column into a CollectionTube, and transfer 750 μl of the ethanol added sample (including any precipitate) to FARB Mini Column . Centrifuge for 1 min and discard the flow-through.
7. Repeat step 6 for rest of the sample.
8. (Optional): To eliminate genomic DNA contamination, follow the steps from 8a. Otherwise, proceed to step 9 directly.

8a. Add 250 μl of Wash Buffer 1 to wash FARB Mini Column. Centrifuge at full speed (14,000 rpm) for 1 min then discard the flow-through.

8b. Add 100 μl of RNase-free DNase 1 solution (0.5U/μl, not provided) to the memberane center of FARB Mini Column. Place the Column on the benchtop for 15 min.

8c. Add 250 μl of Wash Buffer 1 to wash FARB Mini Column. Centrifuge at full speed (14,000 rpm) for 1 min then discard theflow-through.

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

9. Add 500 μl of Wash Buffer 1 to wash FARB Mini Column. Centrifuge for 1 min then discard the flow-through.

10. Wash FARB Mini Column twice with 700 μl of Wash Buffer 2 by centrifuge at full speed (14,000 rpm) for 1 min then discard the flow-through.

    Caution: Make sure that ethanol has been added into Wash Buffer 2 when first open.
11. Centrifuge at full speed (14,000 rpm) for an additional 3 min to dry the column.

    Caution: Important Step! This step will avoid the residual liquid to inhibit the subsequent enzymatic reactions.
12. Places FARB Mini Column into a Elution Tube.
13. Add 50 μl of RNase-free ddH_2O to the membrane center of FARB Mini Column. Stand FARB Mini Column for 1 min.
14. Centrifuge for 1 min to elute RNA.
15. Store RNA at -70°C.

Protocol: (Purification of total RNA from bacteria)

1. Transfer 1 min 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) 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. Incubate at room temperature for 3 min.
   (For Preparation of FARB Buffer (*β*-ME added), See Important Note:3).
6. Centrifuge at full speed (14,000 rpm) for 2 min to spin down insoluble materials and transfer the supernatant to a microcentrifuge tube (not provided).
7. Add 1 Volume of 70% ethanol to the clear lysate and mix by pietting.
8. Follow the Animal Cells Protocol starting from step 6.

Protocol: (Purification of total RNA from 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 xg) for 10 min and discard the supernatant completely.
3. Resuspend the cell pellet in 600 µl sorbitol buffer (not provided) (1M sorbitol; 100 mM EDTA; 0.1 % β-ME). Add 200 U zymolase or lyticase at 30℃ for 30 min.
4. Centrifuge at 7,500 rpm (5,000 xg) 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. Incubate at room temperature for 3 min. (For Preparation of FARB Buffer (β-ME added), See Important Note:3).
6. Centrifuge at full speed (14,000 rpm) 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.

DNA Contamination:

This kit has been optimized for preferential RNA binding, however genomic DNA contamination is almost impossible to avoid during RNA extraction procedures. DNase I-RNase Free may be applied to the binding column according to the protocol instructions. It is necessary to use highly purified DNase. If RNase is present in trace amounts it will result in RNA degradation. It is recommended to apply DNase for sensitive downstream applications, however for many downstream applications it may not be necessary to apply as genomic DNA contamination may be negligible or inconsequential to the application.