



FavorPrep™ Blood/Cultured Cell Total RNA Maxi Kit

-For isolation RNA from human whole blood, animal cells, bacteria, yeast

Cat. No.: FABRK 000-Maxi
FABRK 003
FABRK 003-1

For Research Use Only

Kit Contents:

| Cat. No: | FABRK 000-Maxi (2 preps) | FABRK 003 (10 preps) | FABRK 003-1 (24 preps) |
|--|-----------------------------|-------------------------|---------------------------|
| 10X RL Buffer | 20 ml | 100 ml | 200 ml |
| FARB Buffer | 30 ml | 150 ml | 180 ml × 2 |
| Wash Buffer 1 | 30 ml | 135 ml | 160 ml × 2 |
| Wash Buffer 2 (Concentrate) ^a | 12 ml | 54 ml | 45 ml × 3 |
| RNase-free Water | 1.5 ml × 2 | 12 ml | 30 ml |
| Filter Columns | 2 pcs | 10 pcs | 24 pcs |
| FARB Maxi Columns | 2 pcs | 10 pcs | 24 pcs |
| Elution Tube (50 ml tubes) | 2 pcs | 10 pcs | 24 pcs |
| User Manual | 1 | 1 | 1 |
| Preparation of Wash Buffer 2 by adding ethanol (96~100%) | | | |
| Ethanol volume for Wash Buffer 2 ^a | 48 ml | 216 ml | 180 ml |

Specification:

Principle: maxi spin column (silica matrix)
Operation time: <60 mins
Binding capacity: up to 2000 µg total RNA/column
Column applicability: centrifugation and vacuum
Minimum elution volume: 500 µl

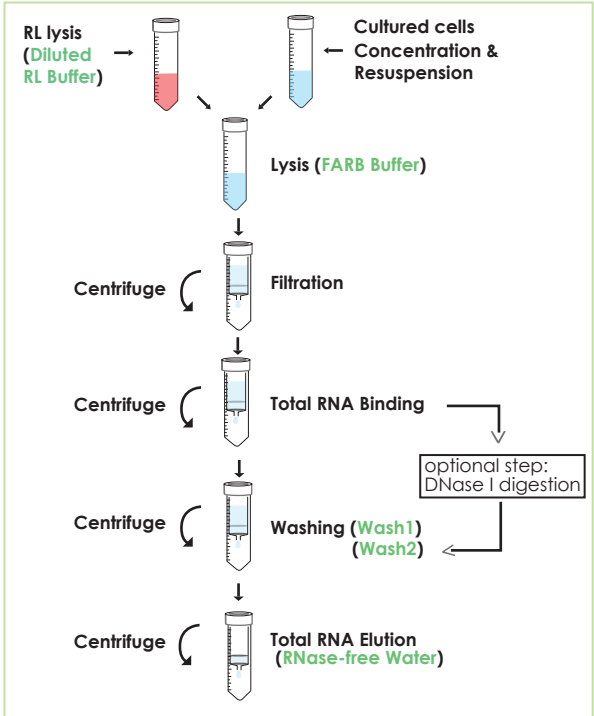
Sample amount and yield

| Sample | Recommended amount of sample used | |
|-------------------|-----------------------------------|--------------------------|
| Human whole blood | 3~10 ml | |
| Animal cells | NIH/3T3 HeLa COS-7 LMH | 5×10 ⁸ cells |
| Bacteria | E. coli B. subtilis | 5×10 ¹⁰ cells |
| Yeast | S. cerevisiae | 5×10 ⁹ cells |

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

Brief Procedure



4. Caution: β-mercaptoethanol (β-Me) is hazardous to human health. Perform the procedures involving β-Me in a chemical fume hood.
5. Add RNase-free ethanol (96~100%) to Wash Buffer 2 at the first use.
6. Use a centrifuge with a swinging bucket rotor for 50 ml tube in all centrifugation steps. The maximum speed should be 4,000-5,000 xg.
7. Prepare RNase-free DNase I reaction buffer (1 M NaCl, 10 mM MnCl₂, 20 mM Tris-HCl, pH 7.0 at 25°C) Kit Contents: and make the final concentration of DNase I to 0.25 U/µl.
8. RL buffer is provided as a 10X concentrate which would must be diluted with sterile deionized water before use.

Protocol: Isolation of Total RNA from Human Whole Blood

Please Read Important Notes Before Starting Following Steps.

Additional requirement: β-Mercaptoethanol and 70% RNase-free ethanol

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 5X volume of diluted RL Buffer with 1X volume of the sample and mix well by inversion.
For example, add 25 ml of diluted RL Buffer to 5 ml of blood sample. For preparation of diluted RL Buffer, See Important Note: 8.
4. Incubate at room temperature for 5 mins. Vortex briefly 2 times during incubation.
5. Centrifuge for 5 mins at 500 xg to pellet cell and discard the supernatant completely.
6. Add 2X volume of diluted RL Buffer to wash the cell pellet by briefly vortexing.
7. Centrifuge for 5 mins at 500 xg to pellet cell and discard the supernatant completely.
8. Add 12.5 ml of FARB Buffer (β-ME added) to the cell pellet and vortex vigorously. Incubate at room temperature for 3 mins to lyse cells completely. (For preparation of FARB Buffer <β-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 mins.
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 Column, centrifuge at full speed for 5 mins. Discard the flowthrough and place the FARB Maxi Column back in 50 ml centrifuge tube.
-The maximum capacity of FARB Maxi Column is 14 ml, repeat Step 12 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 mins. Discard the flowthrough and place the FARB Maxi Column back in 50 ml centrifuge tube.
13b. Add 12.5 ml of 70% ethanol to wash FARB Maxi Column. Centrifuge at full speed for 2 mins. Discard the flowthrough and place the FARB Maxi Column back in 50 ml centrifuge tube.
13c. Add 1 ml of RNase-free DNase I solution (0.25 U/µl, not provided) to the membrane center of FARB Maxi Column. Place the Column on the benchtop for 10 mins.
13d. Add 7 ml of Wash Buffer 1 to wash FARB Maxi Column. Centrifuge at full speed for 2 mins. Discard the flow-through and place the FARB Maxi Column back in 50 ml centrifuge tube.
13e. After DNase I 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 mins. 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 mins.
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 at the first open.
16. Centrifuge at full speed for an additional 10 mins 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 mins.
-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 mins to elute RNA.
20. Store RNA at -70°C.

Protocol: Isolation of Total RNA from Animal Cells
Please Read Important Notes Before Starting Following Steps.

Additional requirement: β-Mercaptoethanol and 70% RNase-free ethanol

1. Pellet up to 5×10⁸ of animal cells by centrifuge at 300 xg for 5 mins. Discard the supernatant completely.
2. Add 14 ml of FARB Buffer (β-ME added) to the cell pellet and vortex vigorously. Incubate at room temperature for 5 mins.
(For preparation of FARB Buffer <β-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 mins.
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 12.

Protocol: Isolation of Total RNA from Bacteria
Please Read Important Notes Before Starting Following Steps.

Additional requirement: β-Mercaptoethanol
70% RNase-free ethanol
37°C water bath or heating block
Lysozyme reaction solution: 10 mg/ml lysozyme; 20 mM Tris-HCl, pH 8.0; 2 mM EDTA; 1.2% Triton

1. Transfer up to 5×10¹⁰ of well-grown bacterial to a centrifuge tube(not provided).
2. Descend the bacterial cells by centrifuge at >3,000 xg for 5 mins and discard the supernatant completely.
3. Resuspend the cell pellet in 1 ml of RNase-free lysozyme reaction solution (10 mg/ml lysozyme; 20 mM Tris-HCl, pH 8.0; 2 mM EDTA; 1.2% Triton) (not provided).
4. Incubate at 37°C for 10 mins.
5. Add 13 ml of FARB Buffer (β-ME added) to the sample and mix well by vortex. Incubate at room temperature for 5 mins. (For preparation of FARB Buffer <β-ME added>, see Important Note: 3)
6. Centrifuge at full speed for 5 mins to spin down insoluble 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 12.

Protocol: Isolation of Total RNA from Yeast
Please Read Important Notes Before Starting Following Steps.

Additional requirement: β-Mercaptoethanol
70% RNase-free ethanol
Lyticase or zymolyase
Sorbitol buffer (1 M sorbitol; 100 mM EDTA; 0.1% β-ME)
30°C water bath or heating block

1. Transfer up to 5×10⁹ yeast cells to a 50 ml centrifuge tube. (not provided)
2. Descend the yeast cells by centrifuge at 500 xg at 4°C for 5 mins and discard the supernatant completely.
3. Resuspend the cell pellet in 2.5 ml of enzymatic lysis buffer (20 mg/ml lyticase or zymolyase; 1 M sorbitol; 100 mM EDTA; 0.1% β-ME) (not provided). Incubate at 30°C for 30 mins.
-Prepare sorbitol buffer just before use.
4. Centrifuge at 500 xg at room temperature for 5 mins to pellet spheroplasts and discard the supernatant completely.
5. Add 14 ml of FARB Buffer (β-ME added) to the sample and mix well by vortexing. Incubate at room temperature for 5 mins.
6. Centrifuge at full speed for 5 mins 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 General Protocol starting from step 12.