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

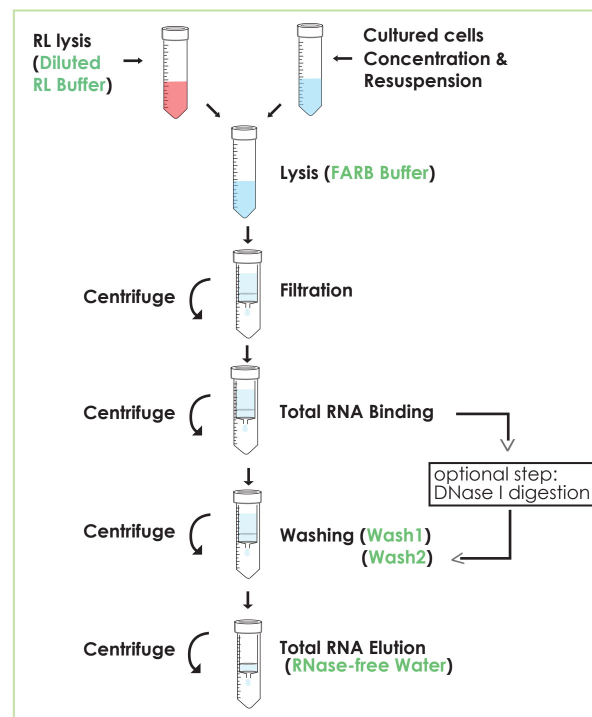
Specification:

Principle: maxi spin column (silica matrix)
Operation time: <60 mins
Binding capacity: up to 2000 µg total RNA/column
Column applicability: centrifugation and vaccum
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

Brief Procedure



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 ß-mercaptoethanol (ß-ME) per 1 ml FARB Buffer before use.
4. Caution: ß-mercaptoethanol (ß-Me) is hazardous to human health. Perform the procedures involving ß-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 x g.
7. Prepare RNase-free DNase I reaction buffer (1 M NaCl, 10 mM $MnCl_2$, 20 mM Tris-HCl, pH 7.0 at 25°C) Kit Contents: and make the final concentration of DNase I to 0.5 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: ß-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 x g 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 x g to pellet cell 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 mins 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 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 Colum, 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 1 ml of RNase-free DNase I solution (0.5 U/µl, not provided) to the membrane center of FARB Maxi Column. Place the Column on the benchtop for 10 mins.
    13c. 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.
    13d. 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: ß-Mercaptoethanol and 70% RNase-free ethanol

1. **Pellet up to 5×10^8 of animal cells by centrifuge at 300 x g for 5 mins. 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 mins.**
   (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 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: ß-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 5×10^{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 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 (ß-ME added) to the sample and mix well by vortex. Incubate at room temperature for 5 mins.** (For preparation of FARB Buffer <ß-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: ß-Mercaptoethanol
70% RNase-free ethanol
Lyticase or zymolyase
Sorbitol buffer (1 M sorbitol; 100 mM EDTA; 0.1% ß-ME)
30°C water bath or heating block

1. **Transfer up to 5×10^9 yeast cells to a 50 ml centrifuge tube. (not provided)**
2. **Descend the yeast cells by centrifuge at 500 x g 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% ß-ME) (not provided). Incubate at 30°C for 30 mins.**
   -Prepare sorbitol buffer just before use.
4. **Centrifuge at 500 x g at room temperature for 5 mins 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 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.**