FavorPrep™ Blood/Cultured Cell Total RNA Mini Kit

-For isolation RNA from human whole blood, animal cells, animal tissues, bacteria, yeast, paraffin fixed sample, fungi and for RNA clean-up

Cat. No.: FABRK 000-Mini
FABRK 001
FABRK 001-1
FABRK 001-2
For Research Use Only

Kit Contents:

Cat. No:	FABRK 000-Mini (4 preps)	FABRK 001 (50 preps)	FABRK 001-1 (100 preps)	FABRK 001-2 (300 preps)
RL Buffer	15 ml	120 ml	240 ml	240 ml x 3
FARB Buffer	3 ml	25 ml	45 ml	130 ml
Wash Buffer 1	3 ml	30 ml	60 ml	170 ml
Wash Buffer 2 (Concentrate)[a]	1.5 ml	15 ml	35 ml	50 ml x 2
RNase-free Water	0.5 ml	6 ml	6 ml	8 ml x 2
Filter Column	4 pcs	50 pcs	100 pcs	300 pcs
FARB Mini Column	4 pcs	50 pcs	100 pcs	300 pcs
Collection Tube	8 pcs	100 pcs	200 pcs	600 pcs
Elution Tube	4 pcs	50 pcs	100 pcs	300 pcs
User Manual	1	1	1	1
Preparation of Wash Buffer 2 by adding ethanol (96~100%)				
Ethanol volume for Wash Buffer 2[a]	6 ml	60 ml	140 ml	200 ml

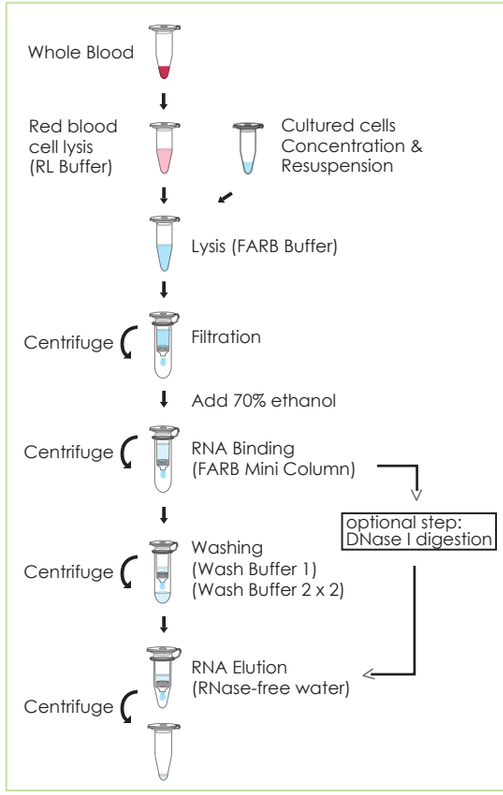
Specification:

Principle: mini spin column (silica matrix)
Operation time: 30~60 mins
Binding capacity: up to 100 µg total RNA/column
Column applicability: centrifugation and vaccum
Minimum elution volume: 40 µl

Sample amount and yield

Sample	Recommended amount of sample used		Yield (µg)
Human whole blood (up to 300 µl)	300 µl		1
Animal cells (up to 5×10^6)	NIH/3T3 HeLa COS-7 LMH	1×10^6 cells	10 15 30 12
Animal Tissue (Mouse/rat) (up to 30 mg)	Embryo Heart Brain Kidney Liver Spleen Lung Thymus	10 mg	25 10 10 30 50 35 15 45
Bacteria	E. coli B. subtilis	1×10^9 cells	60 40
Yeast (up to 5×10^7)	S. cerevisiae	1×10^7 cells	25

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. **Caution: ß-mercaptoethanol (ß-Me) is hazardous to human health. Perform the procedures involving ß-Me in a chemical fume hood.**
4. Add required volume of RNase-free ethanol (96~100%) to Wash Buffer 2 at the first use.
5. All centrifuge steps are done at full speed (~18,000 x g) in a microcentrifuge.
6. Prepare RNase-free DNase I reaction buffer (1 M NaCl, 10 mM $MnCl_2$, 20 mM Tris-HCl, pH 7.0 at 25°C) and make the final concentration of DNase I to 0.5 U/µl.

Protocol: Isolation of Total RNA from Human Whole Blood

Please Read Important Notes Before Starting Following Steps.

Additional requirment: ß-Mercaptoethanol and 70% RNase-free ethanol

1. Red blood cells lysis
   1-1. Add 200~300 µl of anticoagulant-preserved fresh human whole blood to a microcentrifuge tube (1.5 ml or 2.0 ml tube) (not provided). If the sample volume is more than 200 µl, use a 2.0 ml tube as the sample container.
   -Note: Do not overload, too much sample will make cell lysis incompletely and lead to lower RNA yield and purity.
   1-2. Mix 5 volume of RL Buffer with 1 volume of the sample and mix well by inversion.
   1-3. Incubate on ice for 10 mins. Vortex briefly 2 times during incubation.
   1-4. Centrifuge for 1 min at 2,800 x g to form a cell pellet and discard the supernatant completely.
   1-5. Add 600 µl of RL Buffer to resuspend the cell pellet by briefly vortexing.
   1-6. Centrifuge for 1 min at 2,800 x g to form a cell pellet again and discard the supernatant completely.
2. Add 350 µl of FARB Buffer and 3.5 µl of ß-Mercaptoethanol to the cell pellet. Vortex vigorously for 1 min to resuspend the cells completely.
   -Note: If the clump is still visible after vortex, pipet sample mixture up and down to break down the clump.
3. Place a Filter Column to a Collection Tube and transfer the sample mixture to the Filter Column. Centrifuge at full speed (~18,000 x g) for 2 mins.
4. Transfer the clarified supernatant from the Collection Tube to a new microcentrifuge tube (not provided), and measure the volume of the supernatant.
   -Note: Avoid to pipette any debris and pellet when transferring the supernatant.
5. Add 1 volume of 70% RNase-free ethanol and mix well by vortexing.
6. Place a FARB Mini Column to a Collection Tube and transfer the ethanol added sample mixture (including any precipitate) to the FARB Mini Column. Centrifuge at full speed for 1 min, discard the flow-through and return the FARB Mini Column back to the Collection Tube.
7. Optional step: DNase I digestion To eliminate genomic DNA contamination, follow the steps from 7a. Otherwise, proceed to step 8 directly.
   7a. Add 250 µl of Wash Buffer 1 to the FARB Mini Column, centrifuge at full speed for 1 min. Discard the flow-through and return the FARB Mini Column back to the Collection Tube.
   7b. Add 60 µl of RNase-free DNase I solution (0.5 U/µl, not provided) to the membrane center of the FARB Mini Column. Place the column on the benchtop for 15 mins.
   7c. Add 250 µl of Wash Buffer 1 to the FARB Mini Column, centrifuge at full speed for 1 min. Discard the flow-through and return the FARB Mini Column back to the Collection Tube.
   7d. After DNase I treatment, proceed to step 9.
8. Add 500 µl of Wash Buffer 1 to the FARB Mini Column, centrifuge at full speed for 1 min. Discard the flow-through and return the FARB Mini Column back to the Collection Tube.
9. Add 750 µl of Wash Buffer 2 to the FARB Mini Column, centrifuge at full speed for 1 min. Discard the flow-through and return the FARB Mini Column back to the Collection Tube.
   -Note: Make sure that ethanol has been added into Wash Buffer 2 at the first use.
10. Repeat step 9 for one more washing.
11. Centrifuge the FARB Mini Column at full speed for an additional 3 mins to dry the FARB Mini Column.
    -Important Step: This step will avoid the residual liquid to inhibit subsequent enzymatic reaction.
12. Place the FARB Mini Column to an Elution Tube (provided, 1.5 ml microcentrifuge tube).
13. Add 40~100 µl of RNase-free ddH_2O to the membrane center of the FARB Mini Column. Stand the FARB Mini Column for 1 min.
    -Important Step: For effective elution, make sure that RNase-free ddH2O is dispensed on the membrane center and is absorbed completely.
    -Important Step: Do not elute the RNA using RNase-free water less than suggested volume (<40 µl). It will lower the RNA yield.
14. Centrifuge the FARB Mini Column at full speed for 1 min to elute RNA.
15. 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
70% RNase-free ethanol

1. Collect 1~5×10^6 cells by centrifuge at 300 x g for 5 mins at 4°C. Remove all the supernatant.
   -Note: Do not overload, too much sample will make cell lysis incompletely and lead to lower RNA yield and purity.
2. Add 350 µl of FARB Buffer and 3.5 µl of ß-Mercaptoethanol to the cell pellet. Vortex vigorously for 1 min to resuspend the cells completely.
   -Note: If the clump is still visible after vortex, pipet sample mixture up and down to break down the clump.
3. Follow Human Whole Blood Protocol starting from step 3.

Protocol: Isolation of Total RNA from Animal Tissues

Please Read Important Notes Before Starting Following Steps.

Additional requirement: Liquid nitrogen & mortar
Rotor-stator homogenizer or 20-G needle syringe
ß-Mercaptoethanol
70% RNase-free ethanol

1. Weight up to 30 mg of tissue sample. Grind the sample in liquid nitrogen to a fine powder with a mortar and transfer the powder to a new microcentrifuge tube (not provided).
   -Note: Avoid thawing the sample during weighing and grinding.
2. Add 350 µl of FARB Buffer and 3.5 µl of ß-Mercaptoethanol. Homogenize the sample by using a rotor-stator homogenizer or by passing the sample lysate through a 20-G needle syringe 10 times. Incubate at room temperature for 5 mins.
   -Important step: In order to release more RNA from the harder samples, it is recommended to homogenize the sample by using suitable homogenize equipment, for example, with a rotor-stator homogenizer.
3. Follow Human Whole Blood Protocol starting from step 3.

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
2 ml screw centrifuge tube
Lysozyme reaction solution: 10 mg/ml lysozyme; 20 mM Tris-HCl, pH 8.0; 2 mM EDTA; 1.2% Triton
Acid-washed glass beads, 500~700 µm

1. Transfer up to 1×10^9 cells well-grown bacterial culture to a 2 ml screw centrifuge tube.
   -Note: Make sure the amount of total RNA harvested from sample do not excess the binding capacity (100 µg) of column when estimate the sample size. Too much sample will make cell lysis incompletely and lead to lower RNA yield and purity. If RNA amount is hard to determine on some species, using ≤5×10^8 cells as the starting sample size.
2. Descend the bacterial cells by centrifuge at full speed (~18,000 x g) for 2 mins at 4°C. Remove all the supernatant.
3. Add 100 µl of lysozyme reaction solution. Pipet up and down to resuspend the cell pellet and incubate at 37°C for 10 mins.
4. Add 350 µl of FARB Buffer and 3.5 µl of ß-Mercaptoethanol.
5. Add 250 mg of acid-washed glass beads (500 ~700 µm) and vortex vigorously for 5 mins to disrupt the cells.
6. Centrifuge at full speed (~18,000 x g) for 2 mins to spin down insoluble material. Transfer the supernatant to a microcentrifge tube (not provided) and measure the volume of the clear lysate.
   -Note: Avoid pipetting any debris and pellet in the Collection Tube.
7. Follow human Whole Blood Protocol starting from step 5.

Protocol: Isolation of Total RNA from Yeast

Please Read Important Notes Before Starting Following Steps.

Additional requirement: ß-Mercaptoethanol
70% RNase-free ethanol

Enzymatic disruption: Lyticase or zymolyase
Sorbitol buffer (1 M sorbitol; 100 mM EDTA; 0.1% ß-ME)
30°C water bath or heating block

Mechanical disruption: 2 ml screw centrifuge tube
Acid-washed glass beads, 500~700 µm

1. Collect up to 5×10^7 of yeast culture by centrifuge at 5,000 x g for 10 mins at 4°C. Remove all the supernatant.

2A. Enzymtic disruption:
- 2A-1: Resuspend the cell pellet in 600 µl sorbitol buffer (1 M sorbitol; 100 mM EDTA; 0.1% ß-ME) (not provided). Add 200 U zymolyase or lyticase and incubate at 30°C for 30 mins.
  -Note: Prepare sorbitol buffer just before use.
- 2A-2. Centrifuge at 300 x g for 5 mins to pellet the spheroplasts. Remove all the supernatant.
- 2A-3. Add 350 µl of FARB Buffer and 3.5 µl of ß-Mercaptoethanol to the pellet. Vortex vigorously to disrupt the spheroplasts for 1 min. Incubate sample mixture at room temperature for 5 mins.

2B. Mechanical disruption:
- 2B-1. Add 350 µl of FARB Buffer and 3.5 µl of ß-Mercaptoethanol to the pellet and vortex vigorously to resuspend the cells completely.
- 2B-2. Transfer the sample mixture to a 2 ml screw centrifuge tube and add 250 mg of acid-washed glass beads (500~700 µm) and vortex vigorously for 15 mins to disrupt the cells.
- 2B-3. Transfer the clarified supernatant from the 2 ml screw centrifuge tube to a new microcentrifuge tube (not provided), and measure the volume of the supernatant.

3. Follow Human Whole Blood Protocol starting from step 5.

Protocol: Isolation of Total RNA from Paraffin-embedded tissue

Please Read Important Notes Before Starting Following Steps.

Additional requirement: Xylene & ethanol (96~100%)
Liquid nitrogen & mortar
Rotor-stator homogenizer or 20-G needle syringe
ß-Mercaptoethanol
70% RNase-free ethanol

1. Transfer up to 15 mg paraffin-embedded tissue sample to a microcentrifuge tube (not provided).
   -Remove the extra paraffin to minimize the size of the sample slice.
2. Add 0.5 ml xylene, mix well and incubate at room temperature for 10 mins.
3. Centrifuge at full speed for 3 mins. Remove the supernatant by pipetting.
4. Add 0.25 ml xylene, mix well and incubate at room temperature for 3 mins.
5. Centrifuge at full speed for 3 mins. Remove the supernatant by pipetting.
6. Repeat step 4 and step 5.
7. Add 0.3 ml ethanol (96~100%) to the deparaffined tissue, mix gently by vortexing. Incubate at room temperature for 3 mins.
8. Centrifuge at full speed for 3 mins. Remove the supernatant by pipetting.
9. Repeat step 7 and step 8.
10. Follow Animal tissue Protocol starting from step 1 for sample disruption then follow Human Whole Blood protocol starting from step 3.

Protocol: RNA Clean-Up

Please Read Important Notes Before Starting Following Steps.

Additional requirement: Ethanol (96~100%)

1. Trandfer 100 µl of RNA sample to a microcentrifuge tube (not provided).
   -If the RNA sample is less than 100 µl, add RNase-free water to make the sample volume to 100 µl.
2. Add 300 µl of FARB Buffer and 300 µl of RNase-free ethanol (96~100%) and mix well by vortexing.
3. Place a FARB Mini Column to a Collection Tube and transfer the ethanol added sample mixture to the FARB Mini Column. Centrifuge at full speed for 1 min and discard the flow-through and return the FARB Mini Column back to the Collection Tube.
4. Follow Human Whole Blood Protocol starting from step 8.