# FavorPrep™ Total RNA Mini Kit (Blood/Cultured Cell)

# User Manual

**Cat. No.: FABRK 100 (100 Preps)**
**FABRK 300 (300 Preps)**

**For Research Use Only**

# Kit Contents

| Cat. No. / preps | FABRK 100 (100 Preps) | FABRK 300 (300 Preps) |
|---|---|---|
| RBC Lysis Buffer | 200 ml | 500 ml |
| FARB Buffer | 60 ml | 130 ml |
| FART Buffer | 30 ml | 75 ml |
| Wash Buffer 1 | 50 ml | 130 ml |
| Wash Buffer 2** (concentrated) | 25 ml | 50 ml x 2 |
| RNase-free Water | 6 ml | 30 ml |
| FARB Column | 100 pcs | 300 pcs |
| 2 ml Collection Tube | 200 pcs | 600 pcs |

**Add 100 ml / 200 ml of ethanol (96~100%) to Wash Buffer when first open.

# Blood/Cultured Cell Protocol:

**Sample Preparation**
**For Fresh whole human blood:**

i. Collect fresh human blood in an anticoagulant-treat collection tube.

ii. Add 1 ml of RBC Lysis Buffer to an appropriately sized micro-centrifuge tube (1.5 ml or 2.0 ml tube). (not provided).

iii. Add 300 µl of whole human blood and mix by inversion.

iv. Incubate on ice for 10 minutes. Vortex briefly 2 times during incubation.

v. Centrifuge for for 5 minutes at 1,000 x g at 4°C to form a cell pellet and discard the supernatant completely.

vi. Resuspend the pellet with 100 µl of RBC Lysis Buffer by pipetting.

**For Cultured animal cells:**

i. Trypsinize the adherent cells before harvesting.

ii. Transfer the appropriate number of cell (up to 5 x 106) to a 1.5ml microcentrifuge tube (not provided) and centrifuge at 6000 x g for 20 seconds.

iii. Remove the supernatant and resuspend the cells with 100 µl of RBC Lysis Buffer.

# Blood/Cultured Cell Protocol:

**Step 1 –Cell Lysis**

1. **Add 400 µl of FARB Buffer (ß-ME added) to the sample and vortex vigorously. (For preparation of FARB Buffer (ß-ME added), See Important Note: 3)**

2. **Incubate at room temperature for 5 minutes.**

**Step 2 – Binding**

3. **Add 500 µl of 70% ethanol to the sample and shake vigorously (pipetting if there is any precipitate).**

4. **Place a RB Column in a 2 ml Collection Tube.**

5. **Transfer the ethanol-added mixture to the RB Column. Centrifuge for 1 minute at full speed(14,000 rpm or 10,000 x g) and discard the flow-through.**

6. **Repeat step 5 for any rest sample.**

7. **Place the RB Column in a new 2 ml Collection Tube.**

**Optional Step:DNA residue degradation(To eliminate genomic DNA contamination, follow the optional steps.)**

8. **Add 100 µl of DNase I (2 KU/ml) mixed in a reaction buffer {50 mM Tris-HCl (pH 7.5), 10 mM $MnCl_2$, 50 µg/ml BSA at 25°C} to the center of the FARB Column matrix. Place the Column on the benchtop for 10 minutes at room temperature.**

# Blood/Cultured Cell Protocol:

**Step 3 – Washing**

9. **Add 400 µl of Wash Buffer 1 to wash FARB Mini Column. Centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min then discard the flow-through.**

10. **Wash FARB Mini Column twice with 600 µl of Wash Buffer 2 by centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min then discard the flow-through.**
    **--Make sure that ethanol has been added into Wash Buffer 2 when first open.**

11. **Centrifuge at full speed (14,000 rpm or 10,000 x g) for an additional 3 min to dry the column.**
    **--Important Step! This step will avoid the residual liquid to inhibit subsequent enzymatic reaction.**

**Step 4 – Elution**

12. **Place FARB Mini Column to RNase-free 1.5ml microcentrifuge tube.**

13. **Add 50 µl of RNase-free Water to the membrane center of FARB Mini Column. Stand FARB Mini Column for 3 min or until the water has been absorbed by the matrix.**
    **--Important Step! For effective elution, make sure that RNase-free Water is dispensed on the membrane center and is absorbed completely.**

14. **Centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min to elute RNA. Optional Step:DNA residue degradation(To eliminate genomic DNA contamination, follow the optional steps.)**

15. **Add 2 µl of DNase I (2 KU/ml) mixed in a reaction buffer {50 mM Tris-HCl (pH 7.5), 10 mM MnCl2, 50 µg/ml BSA at 25°C} to the final elution sample. Stand for 10 minutes at room temperature.**

**Step Final - Pure RNA**

16. **Store RNA at -70°C.**

# Bacteria Protocol:

**Sample Preparation**
**For Gram-negative bacteria:**

i. Transfer the appropriate number of cell (up to 1 x 109) to a 1.5ml microcentrifuge tube (not provided) and centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 minute, then discard the supernatant.

ii. Vortex the pellet for 30 seconds.

iii. Add 200 µl of FART Buffer and resuspend the pellet by vortex.

iv. Incubate for 5 minutes at room temperature.

**For Gram-positive bacteria:**

i. Transfer the appropriate number of cell (up to 1 x 109) to a 1.5ml microcentrifuge tube (not provided) and centrifuge at 6000 x g for 1 minute, then discard the supernatant.

ii. Add 200 µl of lysozyme buffer (20 mg/ml lysozyme; 20 mM Tris-HCl; 2 mM EDTA; 1% Triton X-100; pH 8.0, prepare fresh lysozyme buffer immediately prior to use) and resuspend the pellet by vortex.

iii. Incubate for 10 minutes at room temperature. During incubation, invert the tube every 2-3 minutes.

**Step 1 –Cell Lysis**

1. Add 300 µl of FARB Buffer (ß-ME added) to the sample and mix well by vortex. Incubate for 5 minutes at room temperature. (For preparation of FARB Buffer (ß-ME added), see Important Note: 3)

2. Centrifuge at full speed (14,000 rpm or 10,000 x g) for 2 minutes to spin down insouble material and transfer the supernatant to a new microcentrifge tube. (not provided)

3. Follow the Blood/Cultured Cell Protocol starting from Step 2 (Binding).

# Fungus Protocol:

**Sample Preparation**

i. **Harvest appropriate number of cell (up to 5 x 107) to a 1.5ml microcentrifuge tube (not provided) and centrifuge at 5000 x g for 10 minute, then discard the supernatant.**

ii. **Add 600 µl of sorbitol buffer (1.2 M sorbitol; 10 mM CaCl2; 0.1 M Tris-HCl pH 7.5; 35mM mercaptoethanol) and resuspend the pellet.**

iii. **Add 200 U of lyticase or zymolase. Incubate for 30 minutes at 30°C.**

iv. **Centrifuge the mixture at 2,000 x g for 10 minutes to harvest the spheroplast, and then remove the supernatant.**

v. **Follow the Bacteria Protocol starting from Step 1 (Cell Lysis).**