FavorPrep™
96-well Total RNA Kit

For high-throughput extraction of total RNA from animal cultural cells and bacterial cultural cells.

User Manual

Cat. No.: FATRE000
FATRE001
FATRE002

For Research Use Only

v.1211-2

Introduction

96-Well Total RNA Kit is designed for high-throughput extraction of total RNA from animal cultural cells and bacterial cultural cells. The method use a specialized chaotropic salt to lyse cells and inactivate RNase, then RNA in chaotropic salt is bonded to glass fiber matrix when the lysis mixture passing through the binding plate. After washing off the contaminants, the purified RNA is eluted by RNase free water. The entire procedure can be completed in one hour without phenol/ chloroform extraction and alcohol precipitation. In the procedure, RNA binding and washing steps could be do on vacuum manifold or by centrifuge. And the eluted RNA is ready to use in differt application.

Quality Control

The quality of 96-Well RNA Kit is tested on a lot-to-lot basis. The purified RNA is checked by agarose gel analysis and quantified with spectrophotometer.

Kit Content

	FATRE000 (1 plate)	FATRE001 (4 plates)	FATRE002 (10 plates)
FARB Buffer	30 ml	120 ml	320 ml
Wash Buffer 1	65 ml	130 ml	500 ml, 135 ml
Wash Buffer 2 (concentrated)	25 ml*	50 ml*	50 ml ** x 2, 25 ml*
RNase-free Water	12 ml	50 ml	100 ml
96-Well RNA binding plate	1 pcs	2 x 2 pcs	5 x 2 pcs
96-Well PCR plate	1 pcs	2 x 2 pcs	5 x 2 pcs
Adhesive film	3 pcs	10 pcs	22 pcs

* Add 100 ml of ethanol (96-100%) to Wash Buffer 2 when first use.
** Add 200 ml of ethanol (96-100%) to Wash Buffer 2 when first use.

Caution

FARB Buffer and Wash Buffer 1 contain irritant agent. Wear gloves and lab coat when handling these buffer.

Important Note

1. Make sure everything is RNase-free when handing RNA.
2. Buffer provided in this system contain irritants. Wear gloves and lab coat when handling these buffer.
3. Pipet a required volume of FARE Buffer to another RNase-free container and add 10 µl of ß-mercaptoethanol (ß-ME) per 1 ml FARE Buffer before use.
4. Add required volumeof ethanol (96~100%) to Wash 2 Buffer when first open.
5. (Optional: step13) Dilute RNase-free DNase 1 in dilution buffer (150mM NaCl, 1 mM MgCl2, 10 mM Tris HCl, pH 7.5) to final conc. = 0.5U/ µl.

RNA Binding

7. Add 250 µl of 70% ethanol to each well of the 96-Well 2 ml plate. Mix the sample completely by shaking (seal with adhesive film) or pipetting.
8. Place a 96-Well RNA Binding Plate on top of the vacuum manifold. (optional) Place a 96-Well 2 ml plate inside to collect waste.
9. Transfer the sample mixture to each well of the 96-Well RNA Binding Plate. Apply vacuum at 10 inches Hg for 5 minutes until wells have emptied.

(Optional)
DNase I treatment (To eliminate genomic DNA contamination)

a. Add 250 µ l of Wash Buffer 1 to each well of the 96-Well RNA Binding Plate. Apply vacuum at 10 inches Hg for 5 minutes until wells have emptied.
b. Add 40 µl of RNase-free Dnase I solution (0.5U/µl, not provided) to the membrane center of each well of the 96-well RNA Binding Plate. Place the Plate on benchtop for 15 min.
c. Add 250 µ l of Wash Buffer 1 to each well of the 96-Well RNA Binding Plate. Apply vacuum at 10 inches Hg for 5 minutes until wells have emptied.
d. After DNase I treatment, proceed to step 11.

Wash

10. Add 500 µl of Wash Buffer 1 to each well of the 96-Well RNA Binding Plate. Apply vacuum at 10 inches Hg for 5 minutes until wells have emptied.
11. Add 500 µl of Wash Buffer 2 (ethanol added) to each well of the 96-well RNA Binding Plate. Apply vaccum at 10 inches Hg for 5 minutes until wells have emptied.
12. Apply vacuum at 10 inches Hg for an additional 10 minutes (or incubate at 60 °C for 10 minutes) to remove residual ethanol

RNA Elution

16. Place a clean 96-well PCR Plate (provided) on top of the 96-Well 2 ml plate. And place the RNA Binding Plate on the clean 96-Well PCR plate.
    (top: RNA Binding Plate, middle: 96-well PCR Plate, bottom: 96-Well 2 ml plate)
17. Add 70 µl of RNase free water in the membrane center of each well of RNA Binding Plate. Stand for 3 minutes until Elution Buffer or water absorbed by the matrix.
18. Place the combined plates in a rotor bucket and centrifuge for 5 min at > 4,000 Xg to elute purified RNA.

Wash

12. Add 500 µl of Wash Buffer 1 to each well of the 96-Well RNA Binding Plate.Place the combined plates in a rotor bucket and centrifuge at 3,500 rpm (1,000 ~1,500 x g) for 5 minutes.
13. Add 500 µl of Wash Buffer 2 (ethanol added) to each well of the 96-well RNA Binding Plate. Place the combined plates in a rotor bucket and centrifuge at 3,500 rpm (1,000 ~1,500 x g) for 5 minutes. Discard the flow-through and place the 96-Well RNA Binding Plate back to top of the 96-Well 2 ml plate.
14. Repeat Step 14 for one more time.
15. Place the combined plates in a rotor bucket and centrifuge at > 4,000 rpm for an additional 10 minutes to remove residual ethanol. Discard the flow-through.

RNA Elution

16. Place a clean 96-well PCR Plate (provided) on top of the 96-Well 2 ml plate. And place the RNA Binding Plate on the clean 96-Well PCR plate.
    (top: RNA Binding Plate, middle: 96-well PCR Plate, bottom: 96-Well 2 ml plate)
17. Add 70 µl of RNase free water in the membrane center of each well of RNA Binding Plate. Stand for 3 minutes until Elution Buffer or water absorbed by the matrix.
18. Place the combined plates in a rotor bucket and centrifuge for 5 min at >4,000 rpm to elute purified RNA.

Vaccum/ Centrifuge Protocol

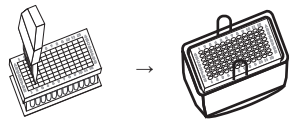
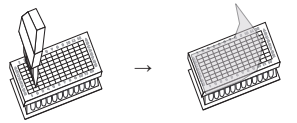
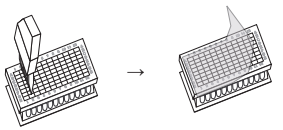
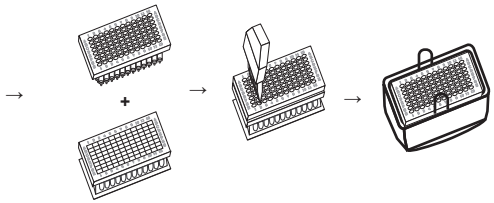
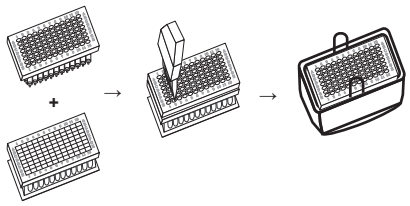
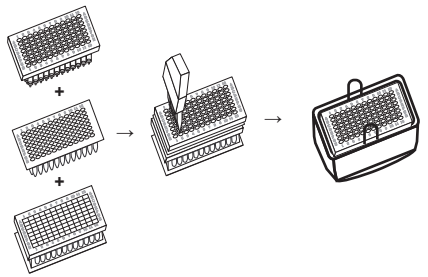
Please Read Important Notes Before Starting The Following Steps.

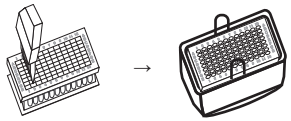
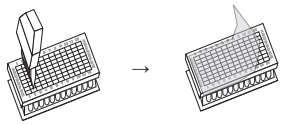
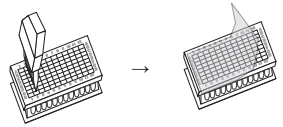
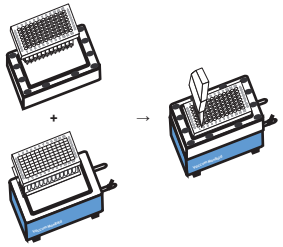
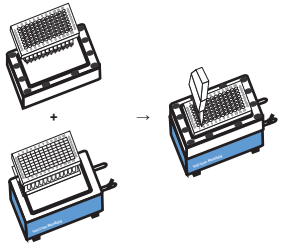
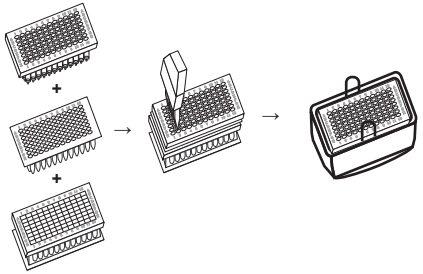
Cell Harvesting

1. Transfer up to 5 x 10^5 cells to each well of a 96-Well 2 ml plate (not provided). Seal with adhesive film.
2. Place the 2 ml 96-Well collection plate in a rotor bucket and centrifuge at 3,500 rpm (1,000 ~1,500 x g) for 5 minutes.
3. Remove cultured medium by pipetting

Cell lysis

4. Add 250 µl of FARB Buffer to each well of the 96-Well 2 ml plate.
5. Lyse the sample by shaking (seal with adhesive film) or pipetting.
6. Incubate at room temperature for 5 minutes until the sample lysate is clear.

Cell Harvesting		1,000 - 1,500 X g 5 min
Cell Lysis		Buffer: 250 µl FARB Buffer Lyse: Shaking or pipetting Incubate: at room temperature, 5 min
RNA Binding		Add 250 µl, 70% ethanol Mix: Shaking or pipetting
		1,000 - 1,500 X g 5 min
RNA Washing		Buffer: Wash1, Wash2 1,000 - 1,500 X g, 5 min Dry membrane >4,000 X g, 10 min
RNA Elution		Buffer: 70 µl, RNase-free Water ,Stand for 5min >4,000 X g, 5min

Cell Harvesting		1,000 - 1,500 X g 5 min
Cell Lysis		Buffer: 250 µl FARB Buffer Lyse: Shaking or pipetting Incubate: at room temperature, 5 min
RNA Binding		Add 250 µl, 70% ethanol Mix: Shaking or pipetting
		vacuum at 10 inches Hg, 5 min
RNA Washing		Buffer: Wash1, Wash2 vacuum at 10 inches Hg, 5 min Dry membrane, vacuum at 10 inches Hg, 5 min
RNA Elution		Buffer: 70 µl, RNase-free Water ,Stand for 5min >4,000 X g, 5min

Centrifuge Protocol

Please Read Important Notes Before Starting The Following Steps.

Cell Harvesting

1. Transfer up to 5 x 10^5 cells to each well of a 96-Well 2 ml plate (not provided). Seal with adhesive film.
2. Place the 2 ml 96-Well collection plate in a rotor bucket and centrifuge at 3,500 rpm (1,000 ~1,500 x g) for 5 minutes.
3. Remove cultured medium by pipetting

Cell lysis

4. Add 250 µl of FARB Buffer to each well of the 96-Well 2 ml plate.
5. Lyse the sample by shaking (seal with adhesive film) or pipetting.
6. Incubate at room temperature for 5 minutes until the sample lysate is clear.

RNA Binding

7. Add 250 µl of 70% ethanol to each well of the 96-Well 2 ml plate. Mix the sample completely by shaking (seal with adhesive film) or pipetting.
8. Place a 96-Well RNA Binding Plate on top of another 96-Well 2 ml plate (not provided).
9. Transfer the sample mixture to each well of the 96-Well RNA Binding Plate.
10. Place the combined plates (96-Well RNA Binding Plate + 96-Well 2 ml plate) in a rotor bucket and centrifuge at 3,500 rpm (1,000 ~1,500 x g) for 5 minutes.
11. Discard the flow-through and place the 96-Well RNA Binding Plate back to top of the 96-Well 2 ml plate.

(Optional)
DNase I treatment (To eliminate genomic DNA contamination)

12a. Add 250 µ l of Wash Buffer 1 to each well of the 96-Well RNA Binding Plate. Place the combined plates in a rotor bucket and centrifuge at 3,500 rpm (1,000 ~1,500 x g) for 5 minutes.
12b. Add 40 µl of RNase-free Dnase I solution (0.5U/µl, not provided) to the membrane center of each well of the 96-well RNA Binding Plate. Place the Plate on benchtop for 15 min.
12c. Add 250 µ l of Wash Buffer 1 to each well of the 96-Well RNA Binding Plate. Place the combined plates in a rotor bucket and centrifuge at 3,500 rpm (1,000 ~1,500 x g) for 5 minutes. Discard the flow-through and place the 96-Well RNA Binding Plate back to top of the 96-Well 2 ml plate.
12d. After DNase I treatment, proceed to step 14.