FAVORGEN

Package Insert
Version 2 .2008.

FavorPrep™

96-Well Total RNA Kit

Cat. No. FATRE 001 (4 plates)

FATRE 002 (10plates)

Store at room temperature (15 - 25℃)

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.

Caution

FARB Buffer and W1 Buffer contain irritant agent. Wear gloves and lab coat when handling these buffer.

Introduction

Name	FATRE001 4 plates	FATRE002 10 plates
FARB Buffer	120 ml	240 ml
Wash 1 Buffer	130 ml	130 ml x 3
Wash 2 Buffer (concentrated)*	50 ml	50 ml x 3
RNase-free ddH_2O	60ml	60 ml x 2
96-Well RNA binding plate	4 pcs	10 pcs
96-Well 350 µl collection plate	4 pcs	10 pcs
Adhesive film	8 pcs	20 pcs

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

References

(1) Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615.

Centrifuge Protocol

Step 1

Cell Harvesting

- Transfer up to 5 x 10^5 cells to each well of a 96-Well 2 ml plate (not provided). Seal with adhesive film.
- 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.
- Remove cultured medium by pipetting.

Step 2

Cell lysis

- Add 200 µl of FARB Buffer to each well of the 96-Well 2 ml plate.
- Lyse the sample by shaking (seal with adhesive film) or pipetting.
- Incubate at room temperature for 5 minutes until the sample lysate is clear.

Step 3

RNA Binding

- Add 200 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.
- Place a 96-Well RNA Binding Plate on top of another 96-Well 2 ml plate (not provided).
- Transfer the sample mixture 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.

Step 4

Wash

- **Add 300 μl of Wash 1 Buffer 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.**
- **Add 600 μl of Wash 2 Buffer (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.**
- **Place the combined plates in a rotor bucket and centrifuge at 3,500 rpm (1,000 ~1,500 x g) for an additional 10 minutes to remove residual ethanol.**

Step 5

RNA Elution

- **Transfer the RNA Binding Plate on a clean 96-Well 350μl collection plate. (provided)**
- **Add 70 μl of preheated Elution Buffer in the center of each well of RNA Binding Plate.**
- **Stand for 3 minutes until Elution Buffer or water absorbed by the matrix. Centrifuge for 5 min at 3,500 rpm to elute purified RNA.**

Vacuum Protocol

Step 1

Cell Harvesting

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

Step 2

Cell lysis

- Add 200 µl of FARB Buffer to each well of the 96-Well 2 ml plate.
- Lyse the sample by shaking (seal with adhesive film) or pipetting.
- Incubate at room temperature for 5 minutes until the sample lysate is clear.

Step 3

RNA Binding

- Add 200 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.
- 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.
- 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.

Step 4

Wash

- **Add 300 µl of Wash 1 Buffer to each well of the 96-Well RNA Binding Plate.**
- **Apply vacuum at 10 inches Hg for 5 minutes until wells have emptied.**
- **Add 600 µl of Wash 2 Buffer (ethanol added) to each well of the 96-well RNA Binding Plate.**
- **Apply vacuum at 10 inches Hg for 5 minutes until wells have emptied.**
- **Apply vacuum at 10 inches Hg for an additional 10 minutes (or incubate at 60 °C for 10 minutes) to remove residual ethanol.**

Step 5

RNA Elution

- **Transfer the RNA Binding Plate on a clean 96-Well 350µl collection plate. (provided)**
- **Add 70 µl of preheated Elution Buffer in the center of each well of RNA Binding Plate.**
- **Stand for 3 minutes until Elution Buffer or water absorbed by the matrix.**
- **Place the combined plates in a rotor bucket and centrifuge at 3,500 rpm for 5 min to elute purified DNA into the 96-Well 350µl collection plate. Seal with Adhesive Film and store 4°C or -20°C.**