User Manual

FavorPrep™ Total RNA Isolation Kit II

(For Research Use Only)

Kit Contents:

Cat. No:	FATRS000 (5 Rxn)	FATRS050 (50 Rxn)	FATRS100 (100 Rxn)
Lysis Buffer	1.5 ml	12.5 ml	25 ml
2 M NaOAc, pH 5.2	150 µl	1.2 ml	2.5 ml
Wash Buffer (Concentrate)[a]	1 ml	7 ml	15 ml
Release Buffer	0.32 ml	3 ml	6 ml
RNA Column	5 pcs	50 pcs	100 pcs
Collection Tube	5 pcs	50 pcs	100 pcs

Preparation of Wash Buffer by adding ethanol (96~100%) and **store at RT.**			
Ethanol volume for Wash Buffer [a]	4 ml	28 ml	60 ml

Specification:

Principle:	mini spin column (silica matrix)
Column Binding Capacity	≤100 µg RNA/column
Sample size:	up to 1×10^6 cultured cells up to 50 mg tissue
Operation time:	30 minutes
Column applicability:	centrifugation and vacuum

Additional requirement to be provided by user

1. Microcentrifuge capable of speed at ~12,000 rpm
2. 1.5 ml microcentrifuge tube
3. 96~100% ethanol
4. Water-Saturated Phenol
5. Chloroform
7. Vortex
8. Water bath or dry bath

Important Notes:

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. Store the kit at room temperature.
3. Caution: phenol and chloroform are hazardous to human health. Perform the procedures involving phenol and chloroform in a chemical fume hood.
4. Add required volume of ethanol (96~100%) to Wash Buffer at the first open. **Store the solution at room temperature.**

Protocol

Read the Important Note before starting the following steps.

Hint: Preheat Release Buffer to 65°C for step 12.

1. Add 200 µl Lysis Buffer into the tube containing up to 100 mg tissue or 1×10^6 cultured cell pellet.
2. Vigorous mixing by vortexing. Incubate at room temperature for 10 minutes.
3. Add 20 µl 2 M NaOAc, pH 5.2.
4. Add 180 µl water-saturated phenol and 40 µl chloroform into the tube, vortex vigorously for 2 minutes.
5. Centrifuge at 12,000 rpm for 3 minutes. Transfer the upper phase into a clean tube.
6. Add 96~100% ethanol (2.33× volume of upper phase) to the upper phase and mix well by shaking vigorously.
   - If the upper phase volume is 220 µl, add 513 µl of 96~100% ethanol to upper phase. The final ethanol concentration of whole mixture will be 70%.
   - Note: Precipitates may be visible after addition of 96~100% ethanol. Resuspend precipitate completely by vigorous shaking and proceed immediately to step 7.
7. Transfer mixture, incoulding precipitate to RNA Column in the Collection Tube. Incubate for 1 minute.
8. Centrifuge at 12,000 rpm for 30 seconds.
9. Add 650 µl Wash Buffer (ethanol added). Incubate for 1 minute.
   - Make sure that ethanol has been added into Wash Buffer at the first open.
10. Centrifuge at 12,000 rpm for 1 minute to completely remove the residue liquid.
11. Put the RNA Column to a clean 1.5 ml tube.
12. Add 30~50 µl Release Buffer (preheated to 65°C) to the center of column. Incubate for 3 minutes.
13. Centrifuge at 12,000 rpm for 3 minutes to recover RNA.