FavorPrep™ After Tri-Reagent RNA Clean-Up Kit

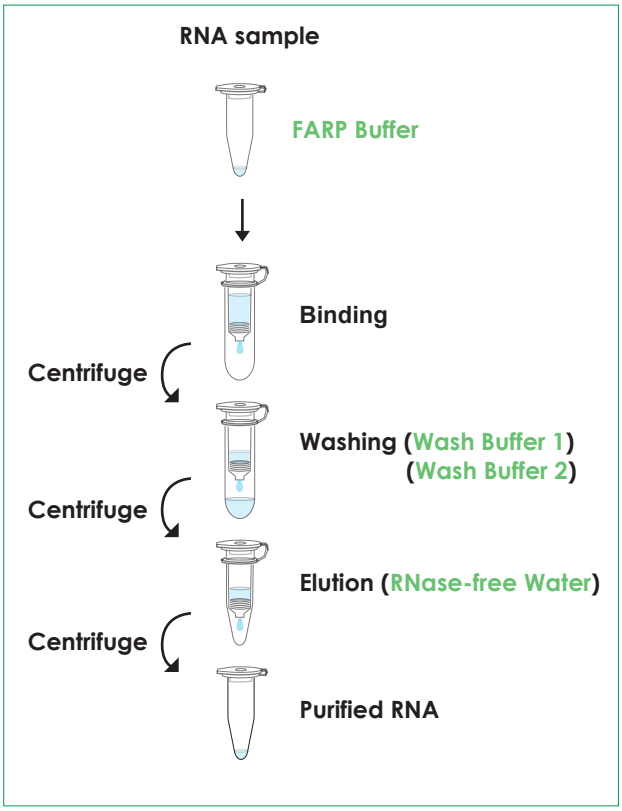
Cat.: FAATR 000, 4 Preps
FAATR 001, 50 Preps
FAATR 001-1, 200 Preps
(For Research Use Only)

Kit Contents:

Cat. No:	FAATR 000 (4 preps)	FAATR 001 (50 preps)	FAATR 001-1 (200 preps)
FARP Buffer	1.8 ml	30 ml	80 ml
Wash Buffer 1	1.5 ml × 2	30 ml	110 ml
Wash Buffer 2 (Concentrate)	1.5 ml	20 ml	35 ml × 2
RNase-free Water	1.5 ml	6 ml	12 ml
FARB Mini Column	4 pcs	50 pcs	200 pcs
Collection Tube	4 pcs	50 pcs	200 pcs
Elution Tube	4 pcs	50 pcs	200 pcs
User Manual	1	1	1
Preparation of Wash Buffer 2 by adding ethanol (96~100%)			
Ethanol volume for Wash Buffer 2	6 ml	80 ml	140 ml

Specification:

Sampling: up to 100 µl of RNA sample or enzymatic reaction mixture
Recovery : 85~95%
Binding capacity: ≤100 µg RNA/column
Volume of eluate : 30~50 µl
Handling Time: Wthin 10 mins



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 buffer.
3. Add ethanol (96~100%) to Wash Buffer 2 at the first open.
4. (Optional step) Dilute RNase-free DNase I in dilution buffer (150 mM NaCl, 1 mM $MgCl_2$, 10 mM Tris HCl, pH 7.5) to final Conc. 0.25 U/µl.
5. All centrifuge steps are done at full speed (14,000 rpm or 10,000 xg) in a microcentrifuge.

General Protocol:

1. Adjust the sample volume to 100 µl with RNase-free water (provided).
   -The maxiimum sample volume is 100 µl.
2. Add 350 µl of FARP Buffer to the sample and vortex vigorously.
3. Add 250 µl of ethanol (96~100%) to the sample mixture and mix well by vortexing.
4. Transfer the entire ethanol added sample (including any precipitate) to FARB Mini Column Set. Centrifuge at full speed (14,000 rpm or 10,000 xg) for 1 min and discard the flow-through.

5. (Optional): To eliminate DNA contamination, follow the steps from 5a. Otherwise, proceed to step 6 directly.

5a. Add 250 µl of Wash Buffer 1 to wash FARB Mini Column. Centrifuge at full speed (14,000 rpm or 10,000 xg) for 1 min then discard the flow-through.

5b. Add 750 µl of 70% ethanol to wash FARB Mini Column. Centrifuge at full speed (14,000 rpm or 10,000 xg) for 1 min then discard the flow-through.

5c. Add 100 µl of RNase-free DNase I solution (0.5 U/µl, not provided) to the membrane center of FARB Mini Column. Place the Column on the benchtop for 15 mins.

5d. Add 250 µl of Wash Buffer 1 to wash FARB Mini Column. Centrifuge at full speed (14,000 rpm or 10,000 xg) for 1 min then discard the flow-through.

5e. After DNase I treatment, proceed to step 7.

6. Add 500 µl of Wash Buffer 1 to wash FARB Mini Column. Centrifugeat full speed (14,000 rpm or 10,000 xg) for 1 min then discard the flow-through.

7. Wash FARB Mini Column twice with 750 µl of Wash Buffer 2 by centrifuge at full speed (14,000 rpm or 10,000 xg) for 1 min then discard the flow-through.
-Make sure that ethanol has been added into Wash Buffer 2 when first open.

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

9. Place FARB Mini Column to Elution Tube (provided).

10. Add 30~50 µl of RNase-free water to the membrane center of FARB Mini Column. Stand FARB Mini Column for 1 min.
-Important Step! For effective elution, make sure that RNase-free Water is dispensed on the membrane center and is absorbed completely.

11. Centrifuge at full speed (14,000 rpm or 10,000 xg) for 2 mins to elute RNA.

12. Store RNA at -70°C.

Troubleshooting

Problem	Possible reasons	Solutions
Little or no RNA eluted	RNA remains on the column	• Repeat elution. • Pre-heat DEPC-water to 70°C prior to elution. • Incubate for 5 mins with water prior to elution.
Degraded RNA	Source	• Follow protocol closely, and work quickly.
	RNase contamination	• Ensure not to introduce RNase during the procedure. • Check buffers for RNase contamination.
Problem in downstream applications	Salt carry-over during elution	• Ensure Wash Buffer 2 has been diluted with 4 volumes of 96~100% ethanol as indicated on bottle. • Repeat wash with Wash Buffer 2.
Abnomal OD reading on A260/A280	DEPC residue remains in DEPC-water	• Use provided RNase-free Water. • Use 10 mM Tris-HCl, not the DEPC water to dilute the sample before measuring purity.