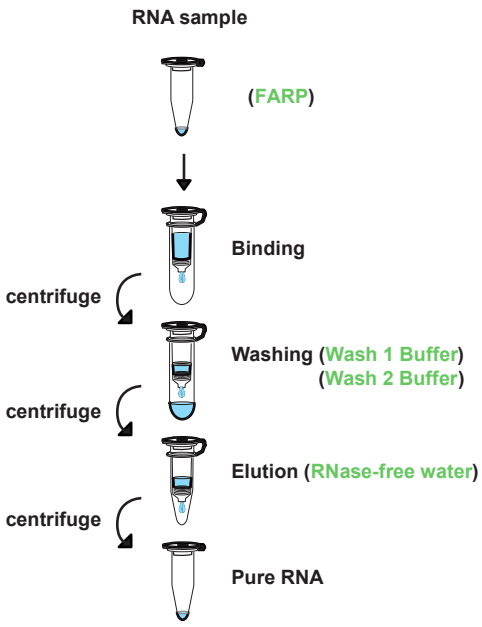
FavorPrep™ After Tri-Reagent RNA Clean-Up Kit

Cat.: FAATR001, 50 Preps
FAATR001-1, 200 Preps
(For Research Use Only) v.1102

Kit Contents:

	FAATR001 (50 preps)	FAATR001-1 (200 preps)
FARP Buffer	30 ml	80 ml
Wash Buffer 1	30 ml	110 ml
Wash Buffer 2 (concentrated)	20 ml*	35 ml** x 2
RNase-free Water	6 ml	12 ml
FARB Mini Column	50 pcs	200 pcs
Collection Tube	50 pcs	200 pcs
Elution Tube	50 pcs	200 pcs



*Add 80 ml ethanol (96-100%) to Wash Buffer 2 when first open.
**Add 140 ml ethanol (96-100%) to Wash Buffer 2 when first open.

Specification:

Sampl Size : up to 100 µl RNA sample or enzymattic reaction mixture

Binding Capacity/ column : up to 100 µg

Recovery : 85-95%.

Handling Time: Within 10 min

Important Notes:

1. Make sure everything is RNase-free when handling RNA.

2. Buffer provided in this kit contain irritants. Wear gloves and lab coat when handling these buffers.

3. Add 6 ml ethanol (96~100%) to Wash Buffer 2 when first open.

4. (For 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. 0.5U/µl.

Genernal Protocol:

Please Read Important Notes Before Starting The Following Steps.

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 x g) 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 x g) for 1 min then discard the flow-through.
5b. Add 100 µl of RNase-free DNase 1 solution (0.5 U/ µl, not provided) to the membrane center of FARB Mini Column. Place the Column on the benchtop for 15 min.
5c. Add 250 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.
5d. After DNase 1 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 x g) for 1 min then discard the flow-through.

7. Wash FARB Mini Columntwice with 750µl 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.

8. 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.

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

10. Add 30~50µl 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 ddH $_2$O is dispensed on the membrane center and is absorbed completely.

11. Centrifuge at full speed (14,000 rpm or 10,000 x g) for 2 min to elute RNA.

12. Store RNA at -70C.

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 min 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 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.

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