# FavorPrep<sup>™</sup> After Tri-Reagent RNA Clean-Up

### **Kit Contents:**

AVORGEN

	FAATR001	FAATROO1-1
	(50 preps)	(200 preps)
FARP Buffer	30 ml	80 ml
Wash Buffer 1	30 ml	110 ml
Wash Buffer 2	20 ml*	35 ml** x 2
(concentrated)		
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.

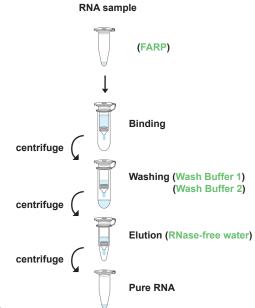
- 3. Add 6 ml ethanol (96~100%) to Wash Buffer 2 when first open.
- 4. (For optional step) Dilute RNase-free DNase I in dilution buffer (150 mM NaCl, 1 mM MgCl<sub>2</sub>, 10 mM Tris HCl, pH 7.5) to final Conc. 0.5U/μl.

### **Genernal Protocol:**

Please Read Important Notes Before Starting The Following Steps.

- 1. Adjust the sample volume to 100  $\mu l$  with RNase-free water (provided). --The maximum sample volume is 100  $\mu l.$
- 2. Add 350  $\mu l$  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.

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- 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 Column *twice* 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<sub>2</sub>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	<ul> <li>Repeat elution.</li> <li>Pre-heat DEPC-water to 70° C prior to elution.</li> <li>Incubate for 5 min with water prior to elution.</li> </ul>	
Degraded RNA	Source	Follow protocol closely, and work quickly.	
	RNase contamination	<ul><li>Ensure not to introduce RNase during the procedure.</li><li>Check buffers for RNase contamination.</li></ul>	
Problem in downstream applications	Salt carry-over during elution	<ul> <li>Ensure Wash Buffer 2 has been diluted with 4 volumes of 100% ethanol as indicated on bottle.</li> <li>Repeat wash with Wash Buffer 2.</li> </ul>	
Abnomal OD reading on A260/A280	DEPC residue remains in DEPC-water	<ul> <li>Use provided RNase-free water.</li> <li>Use 10 mM Tris-HCI, not the DEPC water to dilute the sample before measuring purity.</li> </ul>	