FavorPrep[™] 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.

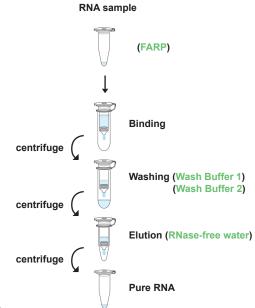
- 3. Add 6 ml ethanol (96~100%) to Wash Buffer 2 when first open.
- 4. (For optional step) Dilute RNase-free DNase I in dilution buffer (150 mM NaCl, 1 mM MgCl₂, 10 mM Tris HCl, pH 7.5) to final Conc. 0.5U/μl.

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 maximum sample volume is 100 $\mu l.$
- 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.

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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 ddH₂O 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-HCI, not the DEPC water to dilute the sample before measuring purity. | |