FavorPrep™ Bacterial RNA Extraction HE Mini Kit

■ Kit Contents

Cat. No.	FABR1030 (4 Preps)	FABR1033 (50 Preps)	FABR1034 (100 Preps)
LR Solution	0.5 ml	6 ml	12 ml
FABR Buffer	1.5 ml x 2	25 ml	50 ml
Wash Buffer 1	1.5 ml x 2	30 ml	60 ml
RNase-Free Water	0.5 ml	6 ml	8 ml
Lysozyme (Lyophilized) ■	5 mg	54 mg	54 mg x 2
FABR HE Columns	4 pcs	50 pcs	50 pcs x 2
Collection Tubes	8 pcs	100 pcs	100 pcs x 2
Elution Tubes	4 pcs	50 pcs	100 pcs
User Manual	1	1	1
Preparation of **Lysozyme**			
Volume of sterile ddH_2O for Lysozyme ■	125 µl	1350 µl	1350 µl

1. All kit components are shipped at room temperature and should be stored at room temperatures between 15~25°C upon receipt, **except Lysozyme**.
2. Store Lysozyme at -20°C upon receipt.

■ Specification

Format/Principle	Spin column (Silica matrix)
Binding Capacity	≤150 µg RNA/Column
Operation Time	<30 mins
Sample Size	≤1 x 10^9 Bacterial cells
RNA yield	≤75 µg
Elution Volume	30 µl

■ Procedure Overview

Centrifuge
5,000 xg, 3 mins

Sample pellet

- Centrifuge to pellet the bacterial sample.
- Discard the supernatant.

- Add 75 µl LR Solution and 25 µl Lysozyme to the sample pellet.
- Incubation at 37˚C for 10 mins.
- Add 400 µl β-ME-FABR mixture.
- Vortex for 5 mins.
- Add 500 µl RNase-Free 70% ethanol.

FABR HE Column

Centrifuge
18,000 xg, 1 min

- Transfer the mixture into the FABR HE Column for RNA binding.

Centrifuge
18,000 xg, 1 min

- Add 500 µl Wash Buffer 1.
- (Optional) DNase I digestion.
- Add 900 µl ethanol (96~100%).

Centrifuge
18,000 xg, 2 mins

- Dry the column membrane.

Centrifuge
18,000 xg, 2 mins

- Add 30 µl RNase-Free Water.
- Stand the column for 5 mins at room temperature.
- Obtain purified RNA.

■ Preparation Before Starting

1. Add the indicated volume of sterile ddH_2O to the Lysozyme tube and dissolve Lysozyme by pipetting to make a 40 mg/ml stock solution. **Aliquot the Lysozyme stock into small portions and store the unused portions at -20˚C.**
2. Additional materials: β-mercaptoethanol (β-ME), RNase-Free 70% and 96~100% ethanol, and DNase I (optional).
3. (Optional) Prepare DNase I working solution following the user guide of FavorPrep™ DNase I Solution (Cat. No. FADNI 050) and make the final concentration of DNase I to 0.25 U/μl.
4. For a fresh preparation of **β-ME-FABR mixture**, premix 4 μl of β-ME and 400 μl of **FABR Buffer** per sample before executing RNA extraction.
5. Set up a water bath or dry bath at 37˚C for the Lysozyme incubation step.
6. **Caution: β-ME is hazardous to human health. Always perform procedures involving β-ME in a fume hood.**

■ General Protocol

1. Transfer up to 1 x 10^9 cultured bacterial cells to a 1.5 ml microcentrifuge tube (not provided).
2. Centrifuge at 5,000 xg for 3 mins to pellet the bacterial sample. Discard the supernatant.
3. Add 75 μl of **LR Solution** and 25 μl **Lysozyme** to resuspend the pellet by pipetting.
4. Incubate the mixture at 37˚C for 10 mins for cell wall disruption.
   - If extremely high-integrity RNA of **Gram-negative bacteria** is required, incubate the mixture at room temperature for 10 mins.
5. Add 400 μl **β-ME-FABR mixture** into the sample mixture.
6. Vortex the sample mixture for 5 mins to lyse the bacterial cells thoroughly.
7. Add 500 μl **RNase-Free 70% ethanol** to the sample mixture. Mix thoroughly by vortexing.
8. Placed a **FABR HE Column** in a **Collection Tube**, then carefully transfer all mixture (including any precipitate) into the FABR HE Column.
9. **(Optional) DNase I digestion.** To eliminate genomic DNA contamination, follow the steps from a.
   a. Add 250 μl of **Wash Buffer 1** to the FABR HE Column, and centrifuge at 18,000 xg for 1 min. Discard the flow-through and place the FABR HE Column in the Collection Tube.
   b. Add 750 μl of **RNase-Free 70% ethanol** to the FABR HE Column, and centrifuge at 18,000 xg for 1 min. Discard the flow-through and place the FABR HE Column in the Collection Tube.

c. Add 60 µl of **RNase-Free DNase I solution** (0.25 U/µl, not provided) to the membrane center of the FABR HE Column. Place the column on the benchtop for 15 mins.

d. Add 250 µl of **Wash Buffer 1** to the FABR HE Column , and centrifuge at 18,000 xg for 1 min. Discard the flow-through and place the FABR HE Column in the Collection Tube.

e. Proceed to step 11.

10. Add 500 µl **Wash Buffer 1** to the FABR HE Column. Centrifuge at 18,000 xg for 1 min then discard the flow-through.
11. Add 900 µl **RNase-Free 96~100% ethanol** to the FABR HE Column. Centrifuge at 18,000 xg for 1 min then discard the flow-through.
12. Centrifuge at 18,000 xg for 2 mins to dry the membrane. Discard the flow-through and the Collection Tube.
13. Place the FABR HE Column in an **Elution Tube**, then add 30 µl **RNase-Free Water** directly onto the membrane. Stand the FABR HE Column for 5 mins.

- **Important step!** For effective elution, ensure that the elution solution is dispensed onto the membrane center and absorbed completely.

14. Centrifuge at 18,000 xg for 2 mins to elute the RNA.