



## FavorPrep™ Bacterial RNA Extraction HE Mini Kit

### ■ Kit Contents

Cat. No.	FABR3030 (4 Preps)	FABR3033 (50 Preps)	FABR3034 (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 Solution	55 µl x 2	650 µl x 2	650 µl x 4
HE Columns	4 pcs	50 pcs	50 pcs x 2
HE Collection Tubes	8 pcs	100 pcs	100 pcs x 2
Elution Tubes	4 pcs	50 pcs	100 pcs
User Manual	1	1	1

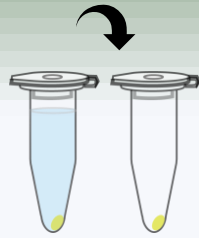
1. All kit components are shipped at room temperature and should be stored at room temperature between 15~25°C upon receipt, **except Lysozyme Solution**.
2. Store Lysozyme Solution at -20°C upon receipt.

### ■ Specification

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

## ■ Procedure Overview

↻ Centrifuge  
5,000 xg, 3 mins



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



- Add 75  $\mu$ l LR Solution and 25  $\mu$ l Lysozyme Solution to the sample pellet.
- Incubation at 37°C for 10 mins.
- Add 400  $\mu$ l  $\beta$ -ME-FABR mixture.
- Vortex for 5 mins.
- Add 500  $\mu$ l RNase-free 70% ethanol.

HE Column

↻ Centrifuge  
18,000 xg, 1 min



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

↻ Centrifuge  
18,000 xg, 1 min



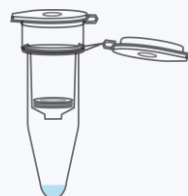
- Add 500  $\mu$ l Wash Buffer 1.

↻ Centrifuge  
18,000 xg, 2 mins



- Add 500  $\mu$ l ethanol (96~100%) and dry the column membrane.

↻ Centrifuge  
18,000 xg, 2 mins



- Add 30  $\mu$ l RNase-Free Water.
- Stand the column for 5 mins.
- Obtain purified RNA.

## ■ Preparation Before Starting

1. Additional materials:  $\beta$ -mercaptoethanol ( $\beta$ -ME), RNase-free 70% and 96~100% ethanol, and DNase I (optional).
2. (Optional) For RNA long-term storage, mix bacterial cells with FavorPrep™ NApreserve Reagent (Cat. No. FNPR1084) as instructed in the user manual.
3. (Optional) Prepare DNase I working solution following the user guide of FavorPrep™ DNase I Solution (Cat. No. FADI2093) and make the final concentration of DNase I to 0.25 U/ $\mu$ l.
4. For a fresh preparation of  **$\beta$ -ME-FABR mixture**, premix 4  $\mu$ l of  $\beta$ -ME and 400  $\mu$ l 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:  $\beta$ -ME is hazardous to human health. Always perform procedures involving  $\beta$ -ME in a fume hood.**

## ■ General Protocol

1. Transfer up to  $1 \times 10^9$  cultured bacterial cells to a 1.5 ml microcentrifuge tube (not provided).
2. Centrifuge at 5,000  $\times g$  for 3 mins to pellet the bacterial sample. Discard the supernatant.
3. Add 75  $\mu$ l of **LR Solution** and 25  $\mu$ l **Lysozyme Solution** 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  $\mu$ l  **$\beta$ -ME-FABR mixture** into the sample mixture.
6. Vortex the sample mixture for 5 mins to lyse the bacterial cells thoroughly.
7. Add 500  $\mu$ l **RNase-free 70% ethanol** to the sample mixture. Mix thoroughly by vortexing.
8. Place an **HE Column** in an **HE Collection Tube**, then carefully transfer all mixture (including any precipitate) into the HE Column. Centrifuge at 18,000  $\times g$  for 1 min then discard the flow-through.
9. **(Optional) DNase I digestion.** To eliminate genomic DNA contamination, follow the steps from a.
  - a. Add 250  $\mu$ l of **Wash Buffer 1** to the HE Column, and centrifuge at 18,000  $\times g$  for 1 min. Discard the flow-through and place the HE Column in the HE Collection Tube.

- b. Add 750  $\mu$ l of **RNase-free 70% ethanol** to the HE Column, and centrifuge at 18,000  $\times$ g for 1 min. Discard the flow-through and place the HE Column in the HE Collection Tube.
  - c. Add 60  $\mu$ l of **RNase-free DNase I solution** (0.25 U/ $\mu$ l, not provided) to the membrane center of the HE Column. Place the column on the benchtop for 15 mins.
  - d. Add 250  $\mu$ l of **Wash Buffer 1** to the HE Column, and centrifuge at 18,000  $\times$ g for 1 min. Discard the flow-through and place the HE Column in the HE Collection Tube.
  - e. Proceed to step 11.
10. Add 500  $\mu$ l **Wash Buffer 1** to the HE Column. Centrifuge at 18,000  $\times$ g for 1 min then discard the flow-through.
  11. Add 500  $\mu$ l RNase-free 96~100% ethanol to the HE Column. Centrifuge for 2 mins to dry the membrane. Discard flow-through and HE Collection Tube.
    - **Important step!** Don't let the tip of the column touch the flow-through. If in doubt, spin again to remove all residual ethanol.
  12. Place the HE Column in an **Elution Tube**, then add 30  $\mu$ l **RNase-Free Water** directly onto the membrane. Stand the HE Column for 5 mins.
    - **Important step!** For effective elution, ensure that the elution solution is dispensed onto the membrane center and absorbed completely.
  13. Centrifuge at 18,000  $\times$ g for 2 mins to elute the RNA.

For more product information, please visit <https://www.favorgen.com/>  
For technical assistance, please email us at [Technical@favorgen.com](mailto:Technical@favorgen.com)

This product is for research use only. It is not intended to be used for therapeutic or diagnostic purposes.