FavorPrep™ Bacterial Genomic DNA Extraction HE Mini Kit

■ Kit Contents

Cat. No.	FABD1030 (4 Preps)	FABD1033 (50 Preps)	FABD1034 (100 Preps)
BGD1 Buffer	2 ml	20 ml	40 ml
BGD2 Buffer	2 ml	30 ml	60 ml
W1 Buffer (Concentrate)▲	0.8 ml x 2	12 ml	24 ml
Elution Buffer	0.5 ml	5 ml	7 ml
Lysozyme (Lyophilized) ■	3 mg	25 mg	50 mg
Proteinase K (Liquid)	150 µl	1050 µl x 2	1050 µl x 4
RNase A Solution	100 µl	1250 µl	1250 µl x 2
FABD 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 **W1 Buffer** and **Lysozyme**			
Volume of ethanol for W1 Buffer▲	1 ml	18 ml	36 ml
Volume of sterile ddH_2O for Lysozyme ■	75 µl	625 µl	1250 µl

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

■ Specification

Format/Principle	Spin column (Silica matrix)
Binding Capacity	≤125 µg DNA/Column
Operation Time	<60 mins
Sample Size	≤5 x 10^9 Bacterial cells ≤2 ml Saliva, Serum or Plasma ≤40 ml Urine ≤1.5 ml Milk 1 swab
DNA yield	≤60 µg
Elution Volume	30 µl

■ Procedure Overview

Sample pellet

- Process the sample as the indication of the **sample preparation steps**.
- Add 312 µl BGD1-Lysozyme mixture to the sample pellet at 37°C for 30 mins.
- Add 35 µl Proteinase K and 470 µl BGD2 Buffer at 60°C for 15 mins.

- Stand at room temperature for 5 mins.
- Add 24 µl RNase A Solution at room temperature for 2 mins.
- Add 135 µl ethanol (96~100%).

FABD HE Column

Centrifuge
12,000 xg, 1 min

- Transfer the mixture into the FABD HE Column for DNA binding.

Centrifuge
12,000 xg, 1 min

- Add 500 µl W1 Buffer (ethanol contained).
- Add 900 µl ethanol (96~100%).

Centrifuge
12,000 xg, 2 mins

- Dry the column membrane.

Centrifuge
12,000 xg, 2 mins

- Add 30 µl Elution Buffer.
- Stand the column for 5 mins at room temperature.
- Obtain purified genomic DNA.

■ Preparation Before Starting

1. Add the indicated volume of sterile ddH_2O to the Lysozyme tube and vortex the solution to ensure complete dissolution as a 40 mg/ml stock solution. **Aliquot the Lysozyme stock into small portions and store the unused portions at -20˚C.**
2. Add the indicated volume of ethanol (96~100%) into the **W1 Buffer**, mix well, and store at room temperature.
3. For a fresh preparation of **BGD1-Lysozyme mixture**, premix 300 µl of **BGD1 Buffer** and 12 µl of **Lysozyme** per sample before executing DNA extraction.
4. Additional materials: 96~100% ethanol.
5. Set up two water baths or dry baths: one at 37˚C for the Lysozyme incubation step, and the other at 60˚C for the Proteinase K incubation and the preheating of the **Elution Buffer** for the elution step.

■ Sample preparation

For Liquid samples (Bacterial Cultures, Biological Fluids, and Water)

1. Transfer up to 5 x 10^9 bacterial cells, 1.5 ml biological fluids, or water samples to a 1.5 ml microcentrifuge tube (not provided).
2. Centrifuge at 14,000 xg for 3 mins. Carefully discard the supernatant. Proceed with the **General Protocol**.

• If sample volume is more than 1.5 ml, repeat the centrifuge step.

For Swab Samples

1. Place the collected swab and resuspend the attached bacterial cell samples into 300 µl of PBS (not provided) in a 1.5 ml microcentrifuge tube (not provided) and mix thoroughly by vortexing.
2. Remove any liquid that remains on the swab.
3. Centrifuge at 14,000 xg for 3 mins. Carefully discard the supernatant. Proceed with the **General Protocol**.

For Milk Samples

1. Transfer up to 1.5 ml of milk sample to a 1.5 ml microcentrifuge tube (not provided).
2. Centrifuge at 14,000 xg for 3 mins. Carefully discard the supernatant.
3. Remove the creamy floats on the top layer after centrifugation and use a paper towel or a cotton swap to remove any white remains on the tube wall. Proceed with the **General Protocol**.

General Protocol

1. Add 312 µl of **BGD1-Lysozyme mixture** and resuspend the pellet by vortexing or pipetting.
2. Incubate the mixture at 37°C for 30 mins for cell wall disruption.
3. Add 35 µl **Proteinase K** and 470 µl **BGD2 Buffer** into the sample mixture. Mix thoroughly.
   - **DO NOT** add Proteinase K directly into BGD2 Buffer.
4. Incubate the mixture at 60°C for 15 mins to lyse the sample.
5. Incubate the sample mixture at room temperature for 5 mins.
6. Add 24 µl **RNase A Solution**. Mix thoroughly and incubate at room temperature for 2 mins.
7. Add 135 µl ethanol (96~100%) to the sample mixture. Mix gently by pipetting or inverting.
8. Placed a **FABD HE Column** in a **Collection Tube**, then carefully transfer all mixture into the FABD HE Column.
9. Centrifuge at 12,000 xg for 1 min. Discard the flow-through and place the FABD HE Column in a new Collection Tube.
10. Add 500 µl **W1 Buffer** (ethanol contained) to the FABD HE Column. Centrifuge at 12,000 xg for 1 min then discard the flow-through.
11. Add 900 µl ethanol (96~100%) to the FABD HE Column. Centrifuge at 12,000 xg for 1 min then discard the flow-through.
12. Centrifuge at 12,000 xg for 2 mins to dry the membrane. Discard the flow-through and the Collection Tube.
13. Place the FABD HE Column in an **Elution Tube**, then add 30 µl prewarmed Elution Buffer or ddH_2O (pH 7.5~9.0) directly onto the membrane. Stand the FABD 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 12,000 xg for 1 min to elute the DNA.