

# FayorPrep™ 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) ▲	$0.8  \text{ml}  \times 2$	12 ml	24 ml
Elution Buffer	0.5 ml	5 ml	7 ml
Lysozyme Solution	55 µl	650 µl	650 µl x 2
Proteinase K (Liquid)	150 µl	1050 µl × 2	1050 µl × 4
RNase A Solution	100 µl	1250 µl	1250 µl × 2
HE Columns	4 pcs	50 pcs	50 pcs × 2
HE Collection Tubes	8 pcs	100 pcs	100 pcs × 2
Elution Tubes	4 pcs	50 pcs	100 pcs
User Manual	1	1	1
Preparation of <b>W1 Buffer</b> by adding 96~100% ethanol.			
Volume of Ethanol for W1 Buffer ▲	1 ml	18 ml	36 ml

- 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 Solution**.
- 2. Store RNase A Solution and Lysozyme Solution 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 <sup>9</sup> Bacterial cells ≤2 ml Saliva, Serum or Plasma ≤40 ml Urine ≤1.5 ml Milk 1 swab
DNA yield	≤60 µg
Elution Volume	30 μΙ

## **■** 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.

Centrifuge 12,000 xg, 1 min



- 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%).

HE Column



Centrifuge 12,000 xg, 1 min

• Transfer the mixture into the HE Column for DNA binding.





• Add 500 µl W1 Buffer (ethanol contained).

Centrifuge 12,000 xg, 2 mins



 Add 500 µl ethanol (96~100%) and dry the column membrane.

Centrifuge 12,000 xg, 2 mins



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

## **■ Preparation Before Starting**

- 1. Add the indicated volume of ethanol (96~100%) into the **W1 Buffer**, mix well, and store at room temperature.
- 2. For a fresh preparation of **BGD1-Lysozyme mixture**, premix 300 µl of **BGD1 Buffer** and 12 µl of **Lysozyme Solution** per sample before executing DNA extraction.
- 3. Additional materials: 96~100% ethanol.
- 4. 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  $5 \times 10^9$  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  $\mu$ l 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 300 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. Place an **HE Column** in an **HE Collection Tube**, then carefully transfer all mixture into the HE column.
- 9. Centrifuge at 12,000 xg for 1 min. Discard the flow-through and place the HE Column in a new HE Collection Tube.
- 10. Add 500 µl **W1 Buffer** (ethanol contained) to the HE column. Centrifuge at 12,000 xg for 1 min then discard the flow-through.
- 11. Add 500 µl 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 prewarmed Elution Buffer or ddH<sub>2</sub>O (pH 7.5~9.0) 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 12,000 xg for 1 min to elute the DNA.

