# Instructions for Use

Version: 1.0.1

## Bacteria Genomic DNA Kit (No RNase A)

**Catalog No.:** abx098240

**Size:** 50 rxns

**Storage:** Store RNase A at -20 °C for up to two years, and the other components at room temperature (15-25 °C) for up to one year.

### Introduction

Abbexa's Bacteria Genomic DNA Kit uses lysozyme and moderate lysis buffer to lyse cells. Proteinase K is used for protein digestion and RNase A used for RNA digestion. DNA is specifically bound to a silica-based column in a hypersaline environment, and DNA is eluted by a low salt and high pH solution. This kit is suitable for isolating high quality genomic DNA from gram-positive and gram-negative bacteria. The isolated genomic DNA is suitable for PCR, restriction enzyme digestion and southern blotting.

### Kit Components

**Reagent**

- Resuspension Buffer: 12 ml
- Lysis Buffer: 6 ml
- Binding Buffer: 10 ml
- Clean Buffer: 55 ml
- Wash Buffer: 12 ml
- Elution Buffer: 25 ml
- Proteinase K (20 mg/ml): 1 ml
- Genomic Spin Columns with Collection Tubes: 50

**Material Required But Not Provided**

- RNase A (10 mg/ml)
- 96-100% ethanol
- 70% ethanol
- Lysozyme
- Glass beads
- Pipettes and pipette tips
- Centrifuge and centrifuge tubes
- Homogenizer
- Water bath or incubator



## Protocol

### Reagent Preparation

- **Working Binding Buffer solution:** Dilute the Binding Buffer with 96-100% ethanol to a ratio of 2:3 (i.e. add 10 ml of Binding Buffer into 15 ml of 96-100% ethanol to form 25 ml of Working Binding Buffer solution).
- **Working Wash Buffer solution:** Dilute the Wash Buffer with 96-100% ethanol to a ratio of 1:4 (i.e. add 12 ml of Wash Buffer into 48 ml of 96-100% ethanol to form 60 ml of Working Wash Buffer solution).
- **Working Lysozyme solution:** Dissolve 4 mg of lysozyme in 200 µl of Resuspension Buffer.

### Sample Preparation

Samples should have a gram-positive or gram-negative bacteria cell count of ≤ $10^9$. Samples which contain a bacteria cell count above this may result in incomplete lysis.

- **Lysis of gram-negative bacteria:**

  1. Transfer 1 ml of overnight culture gram-negative bacteria to 1.5 ml centrifuge tube, and centrifuge at 12,000 × g for 1 minute. Discard the supernatant.
  2. Add 100 µl of Lysis Buffer and 20 µl of Proteinase K to the pellet.
  3. Resuspend the bacteria by vortexing. Incubate at 55 °C for 15 minutes. The solution should be clear after incubation, if not, extend the incubation time to 30 minutes, vortexing every 5 minutes.

- **Lysis of gram-positive bacteria:**

  1. Transfer 1 ml of overnight culture gram-negative bacteria to 1.5 ml centrifuge tube, and centrifuge at 12,000 × g for 1 minute. Discard the supernatant.
  2. *Gram-positive coccus only:* Resuspend the pellet in 500 µl of 70% ethanol, then put on ice for 20 minutes. Centrifuge at 10,000 × g for 1 minute, then discard the supernatant.

     *Actinomyces only:* Use glass beads to break the hyphae clump. Centrifuge at 10,000 × g for 1 minute then discard the supernatant.
  3. Add 200 µl of Working Lysozyme solution (dissolved in Resuspension Buffer) to the tube. Incubate at 37 °C for at least 60 minutes. The incubation time can be extended to up to 3 hours, depending on the amount of bacteria. Centrifuge at 10,000 × g for 1 minute, then discard the supernatant.
  4. Add 100 µl of Lysis Buffer and 20 µl of Proteinase K to the pellet. Resuspend the bacteria by vortexing. Incubate at 55 °C for 15 minutes. The solution should be clear after incubation, if not, extend the incubation time to 30 minutes, vortexing every 5 minutes.

**Assay Procedure**

Use sterile tubes and pipette tips to avoid DNase contamination.

1. Add 20 µl of RNase A to the tube. Mix and allow to stand at room temperature for 2 minutes.
2. Add 400 µl of Working Binding Buffer solution (with ethanol) to the tube and vortex for 30 seconds. A white precipitate or transparent gelatinous matter may be visible at this stage, this does not affect DNA extraction.
3. Transfer the entire contents of the tube to a spin column. Centrifuge at 12,000 × g for 30 seconds. Discard the flow-through.
4. Add 500 µl of Clean Buffer to the column. Centrifuge at 12,000 × g for 30 seconds. Discard the flow-through.
5. Repeat Step 4 above once more.
6. Add 500 µl of Working Wash Buffer solution (with ethanol) to the column. Centrifuge at 12,000 × g for 30 seconds. Discard the flow-through.
7. Repeat Step 6 above once more.
8. Centrifuge at 12,000 × g for 2 minutes to remove residual Working Wash Buffer solution.
9. Place the spin column in a sterile 1.5 ml microcentrifuge tube. Add 50-200 µl of Elution Buffer (preheated to 65 °C to increase DNA yield) or sterile distilled water (pH > 7.0) to the center of the column. Allow to stand at room temperature for 2 minutes. Centrifuge at 12,000 × g for 1 minute to elute the genomic DNA.
10. Repeat Step 9 above once more. The purified DNA can be stored at -20 °C for long-term storage.