EasyPure® Bacteria Genomic DNA Kit

Cat. No. EE161

Storage: RNase A at -20°C for two years; others at room temperature (15-25°C) for one year.

Description

EasyPure® 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 silica-based column in hypersaline condition, and DNA is eluted by 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 DNA is suitable for PCR, restriction enzyme digestion, and Southern blot.

• Fast: the whole process can be completed in 50 minutes

• High yield: DNA yield up to 20 μg

Kit Contents

Component	EE161-01 (50 rxns)
	EE161-11 (50 rxns)
Resuspension Buffer11 (RB11)	12 ml
Lysis Buffer11 (LB11)	6 ml
Binding Buffer11 (BB11)	10 ml
Clean Buffer 11 (CB11)	55 ml
Wash Buffer 11 (WB11)	12 ml
Elution Buffer (EB)	25 ml
RNase A (10 mg/ml)	1 ml (EE161-01)
	0 (EE161-11)
Proteinase K (20 mg/ml)	1 ml
Genomic Spin Columns with Collection Tubes	50 each

Sample requirement

Gram-positive or Gram-negative bacteria cells ≤10^9

Procedures

Before starting, adding appropriate volume of 96-100% ethanol to BB11 and WB11.

	BB11	WB11
50 rxns	15 ml	48 ml

• Lysozyme will be supplied by users. Prepare fresh lysozyme/RB11 mix for each use (4 mg lysozyme/ 200 μl RB11)

• Prepare 70% ethanol for extraction of Gram-positive coccus; prepare glass bead for breaking Actinomyces hyphae clump.

All centrifugation steps are carried out at room temperature.

1. Material treatment

Lysis of Gram-negative Bacteria

(a) Transfer 1 ml of overnight Gram-negative bacteria to a 1.5 ml tube and centrifuge at 12,000×g for 1 minute. Discard the supernatant.

(b) Add 100 μl of LB11 and 20 μl of Proteinase K into the tube. Resuspend the bacteria by vortexing.

(c) Incubate at 55°C for 15 minutes. (Solution should be clear after incubation. If not, extend the incubation time to 30 minutes, vortex for every 5 minutes.)

Lysis of Gram-positive Bacteria

(a) Transfer 1 ml of overnight Gram-positive bacteria to a 1.5 ml tube and centrifuge at 12,000×g for 1 minute. Discard the supernatant.
(Note: when extract Gram-positive coccus, resuspend it with 500 µl of 70% ethanol, incubate on ice for 20 minutes and then centrifuge at 10,000×g for 1 minute, discard the supernatant, then process to step (b). When extract Actinomyces, use glass bead to break the hyphae clump, centrifuge at 10,000×g for 1 minute, discard the supernatant, then process to step (b).)

(b) Resuspend the bacteria by adding 200 µl of RB11 (containing 4 mg lysozyme) to the tube. Incubate at 37°C for at least 60 minutes (Note: the incubation time can be extended to 3 hours if large amount of bacteria is used), and centrifuge at 10,000×g for 1 minute. Discard the supernatant.

(c) Add 100 µl of LB11 and 20 µl of Proteinase K into the tube. Resuspend the bacteria by vortexing.

(d) Incubate at 55°C for 15 minutes. (Solution should be clear after incubation. If not, extend the incubation time to 30 minutes, vortex for every 5 minutes.)

2. Add 20 µl of RNase A to the tube, mix and incubate at room temperature for 2 minutes.
3. Add 400 µl of BB11 (check to make sure 96-100% ethanol has been added) and vortex for 30 seconds. (White flocculent precipitate or transparent gelatinous matter may present in this step, this would not affect DNA extraction)
4. Transfer the entire contents to a spin column, centrifuge at 12,000×g for 30 seconds, discard the flow-through.
5. Add 500 µl of CB11, centrifuge at 12,000×g for 30 seconds, and discard the flow-through.
6. Repeat step 5 once.
7. Add 500 µl of WB11 (check to make sure 96-100% ethanol has been added), centrifuge at 12,000×g for 30 seconds, discard the flow-through.
8. Repeat step 7 once.
9. Centrifuge at 12,000×g for 2 minutes to remove residual WB11.
10. Place the spin column in a sterile 1.5 ml microcentrifuge tube. Add 50-200 µl of Elution Buffer (preheated to 65°C) or sterile, distilled water (pH >7.0) to the center of column. Incubate at room temperature for 2 minutes. Centrifuge at 12,000×g for 1 minute to elute genomic DNA.
11. Repeat step 10 once. Store the isolated DNA at -20°C.

Notes

- To avoid incomplete lysis, do not use too much starting materials.
- Use sterile tubes and pipette tips to avoid contaminations.

FOR RESEARCH USE ONLY