LaboPass ™ Bacteria Mini

Introduction

LaboPass ™ Bacteria Kit provides a simple and fast method for the isolation of total DNA from up to Gram positive / gram negative 3~5 x 10^8 cultured Bacteria cells. The purified DNA using this kit is ready for applications such as PCR, Southern blotting, and restriction enzyme assay.

Storage Conditions

All components of LaboPass ™ Bacteria Kit, except Proteinase K solution and Lysozyme solution, should be stored in room temperature(15-25℃). Proteinase K solution (20 mg/ml) can be stored for 2 months at 4℃ without any decrease in activity. But storage at -20℃ with usable aliquots is recommended for prolonged preservation. Also, Lysozyme solution store at -20℃. Under cool ambient conditions, a precipitate may form in buffer TB. In such a case, heat the bottle at 60℃ to dissolve. LaboPass ™ Bacteria Kit is guaranteed for 1 year after delivered to an end user.

Kit Contents

Cat. No.	CMBA0111	CMBA0112	CMBA0115
No. of preparation	50	200	1000
Spin column	50	200	1000
Collection tube	150	600	3000
Buffer GL	5ml	10ml	10mlx5
Buffer TL	15ml	60ml	60mlx5
Buffer TB	24ml	100ml	100mlx5
Buffer BW(conc.)	19.6ml	76.1ml	76.1mlx5
Buffer NW(conc.)	10.5ml	32.3ml	32.3mlx5
Buffer AE	15ml	60ml	60mlx5
Proteinase K solution (20mg/ml)(1)	1.1ml	1.1mlx4	1.1mlx20
Lysozyme solution (100mg/ml)(1)	1.1ml	1.1mlx2	1.1mlx10

(1) Avoid repeated freezing and thawing. Once thawed, store at 4℃ for a long time..

* All Solution (Buffer) is available to purchase separately.

Little copurification with RNA

LaboPass ™ Bacteria spin column has very stronger affinity to DNA than RNA. Although RNase is not treated, the percentage of RNA from total prepared nucleic acid will be very low even with a transcriptionally active tissue, such as liver or pancreas.

However, if RNA-free DNA is required, RNase should be treated.

Protocols for Cultured Bacteria Cells

Before experiment

- Prepare the water bath to 56℃

- Equilibrate buffer AE to room temperature

- Add appropriate volume of absolute ethanol to Buffer BW and NW before first use.

- All centrifugation should be performed at room temperature.

- Mix Buffer TL and TB thoroughly by shaking before use.

- Buffer TB may precipitate at cool ambient temperature. If so, dissolve it in 60℃ water bath.

1. Transfer 1-2 ml cultured bacteria cell in 1.5 ml tube

Pellet cultured bacterial cell by centrifugation for 2 min at 10,000 X g in a tabletop centrifuge. Discard the supernatant as much as possible.

Use the appropriate volume of bacterial cultures; 1-3 ml. Bacterial culture should be grown for 12 to 16 hours in liquid media (eg. LB). Higher culture volumes can cause reduction of lysis efficiency and overload of a spin column, resulting in unsatisfactory yields.

2. A. Gram negative bacteria : go to protocol 4

B. Gram positive bateria : Resuspend pelleted bacterial cells thoroughly in 40 ul of GL

3. Add 10 ul of Lysozyme solution (100 mg/ml) and mix by tapping or vigorously vortex. Incubate resuspended bacterial cell for 15 min at 37℃

For completely break cell wall, mix 5 -6 time during incubation by inverting tube

→ if large of Bacteria pellet, increase resuspension volume.

(ex) 3 – 5 ml of Cultured bacteria cell: GL 90 ul + Lysozyme 10 ul

Use two separate column

4. Add 200 µl of TL buffer, 20 µl of Proteinase K solution and mix by vortex.

Incubate the lysate for 10 min at 56℃.

Longer incubation will not affect DNA recovery.

5. Check!! If Buffer TB precipitates, pre-heat in a 60℃ water bath to dissolve completely.

4. Spin down the tube briefly to remove any drops from inside of the lid.

6. Optional: If RNA-free DNA is required, add 4㎕ of RNase solution (100mg/ml, not provided), vortex to mix thoroughly, and incubate for 2 min at room temperature.

LaboPass ™ Bacteria spin column has the very stronger affinity to DNA than RNA. Unless RNase is treated, RNA occupies very small portion of eluates. RNA may inhibit some downstream enzymatic reactions, but will not inhibit PCR.

7. Add 400㎕ of Buffer TB. Immediately vortex the tube to mix thoroughly. Spin down the tube briefly to remove any drops from inside of the lid.

8. Apply the mixture to the spin column. Centrifuge for 1 min at 6,000 xg above(>8,000 rpm). Replace the collection tube with new one. (provided)

If more than 700㎕ of mixture volume is processed, apply the mixture twice; apply 700㎕ of the mixture, spin down, discard the flow-through, reinsert empty collection tube, and repeat the steps again until all of the mixture is applied to the spin column. If the mixture has not passed completely through the membrane, centrifuge again at full speed until all of the solution has passed through. Centrifuge at maximum speed will not affect the DNA recovery.

9. Add 700 μℓ of Buffer BW. Centrifuge for 1 min at 6,000 xg above(>8,000 rpm). Replace the collection tube with new one.(provided)

There is no need to increase the volume of Buffer BW and NW for larger sample.

10. Add 500μℓ of Buffer NW. Centrifuge for 3 min at 14,000 xg (full speed). Place the spin column in a clean 1.5 ml tube.(not provided)

Care must be taken at this step for eliminating the carryover of buffer NW. If carryover of buffer NW occurs, centrifuge again for 1 min at full speed with the empty collection tube(not provided) before transferring to the new 1.5 ml tube.

11. Add 200μℓ of Buffer AE or distilled water. Incubate for 2 min at room temperature. Centrifuge at 6,000 xg above(>8,000 rpm) for 1 min.

Ensure that the buffer AE or distilled water is dispensed directly onto the center of spin column membrane for optimal elution of DNA. If higher concentration of eluate is needed or starting amount of sample is very small, elution volume can be decreased to 50 μℓ minimum. However, the small volume of elution buffer will decrease the total amount of DNA recovery. For long-term storage, eluting in buffer AE is recommended. But, EDTA included in the buffer AE may inhibit subsequent enzymatic reactions, so you can avoid such latent problems by using distilled water or Tris-Cl (>pH8.5). When using water for elution, check the pH of water (>pH7.0) before elution.

Contact us ;

Internet : www.cosmogenetech.com

E-mail : labopass@cosmogenetech.com

Tel : 82-2-922-7307

Fax : 82-2-922-7307