Genorise® Bacterial DNA Extraction Kit 1000

This kit is to isolate total DNA including genomic and mitochondrial DNA from 1 x 10^9 Bacterial cells and is for 50 applications. This kit can significantly improve quality and quantity of DNA and is more cost-effective than the similar products.

Materials provided in the kit:
100 ml 5 x Lysis Solution
250 ml Protein Precipitation Solution
250 ml DNA Hydration Solution

Materials required but not provided in the kit:
Proteinase K, prepare at 20 mg/ml and store at -20 ºC
Isopropanol (2-propanol)/Ethanol

Protocol

Cell Lysis

1. Add 0.5 ml of Lysis Solution and 3 µl of Proteinase K solution (20 mg/ml) to 10^9 Bacterial cells (pellet) in a 1.7 ml microcentrifuge tube and resuspend completely by a pipette, and vortex for 30 sec.
2. Incubate at 55 °C for 1 hr or overnight until the cell lysate becomes completely clear.

Protein precipitation

1. Cool sample to room temperature by placing on ice for 1 min.
2. Add 0.2 ml of Protein Precipitation Solution to the lysate.
3. Vortex samples at high speed for 30 sec and place sample into an ice bath for 5 min.
4. Centrifuge at 15,000 x g for 5 min.

DNA Precipitation

1. Pour the supernatant containing DNA into a new 1.7 ml microcentrifute tube.
2. Centrifuge at 15,000 x g for 5 min.
3. Pour the supernatant to a new microcentrifuge tube; repeat step 1 and 2 until no pellet is seen.
4. Pour the supernatant containing DNA into a new 1.7 ml microcentrifute tube containing 0.7 ml 100% isopropanol.
5. Mix the samples by inverting 50 times and incubate at room temperature for 10 min.
6. Centrifuge at 13,000 x g for 5 min.
7. Pour off the supernatant and drain the tube briefly on clean absorbent paper. Add 1 ml of 70% ethanol and invert the tube several times to wash the DNA pellet.
8. Centrifuge at 13,000 x g for 5 min, carefully pour off the ethanol and do not lose the DNA pellet.

9. Invert and drain the tube on clean absorbent paper, completely remove the remaining liquid, and finally allow airing dry 5 min.

DNA Hydration

1. Add 50 µl DNA Hydration Solution.
2. Resuspend the DNA pellet by pipette for 5 times and rehydrate DNA by incubation for 10 min at room temperature.
3. Vortex briefly and pulse spin before use, and store the DNA at -20 °C.

Protocol without Proteinase K

Cell Lysis

1. Add 0.5 ml of Lysis Solution to 10^9 Bacterial cells (pellet) in a 1.7 ml microcentrifuge tube and resuspend completely by a pipette, and vortex for 30 sec.
2. Incubate at 65 °C for 1 hr or overnight until the cell lysate becomes completely clear.

The rest of protocol is the same as above.

Note

1. Following DNA precipitation, if you see a pellet with a color other than white, we recommend you repeat this protocol with a start volume of 0.5 ml of DNA isolate with only 1 hr cell lysis.
2. Air drying of DNA pellet should not exceed 5 min.