Magnetic Bacterial DNA Kit

Cat.No.: HYC265

Product Description

This kit delivers a simple and rapid workflow for DNA extraction from Gram-positive and Gram-negative bacteria. It adopts high-binding-capacity nano-magnetic particle purification technology. Bacterial cells are lysed and digested synergistically by lysozyme and proteinase to release DNA into the lysate. Binding buffer and magnetic particles are added to adsorb DNA, while proteins remain unbound and are discarded. DNA-bound magnetic particles are washed with wash buffer to remove proteins and other contaminants, followed by ethanol-containing wash solution to eliminate residual salts. Finally, purified DNA is eluted with low-salt EB buffer. The eluted DNA can be directly applied to PCR and other downstream assays.

Components

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Magnetic Microspheres	1.2 mL	2.5 mL
Lysozyme	90 mg	180 mg
Proteinase K	12 mg	24 mg
Protease Dissolve Buffer	5 mL	15 mL
Buffer STE	30 mL	60 mL
Buffer SDS	1.5 mL	3 mL
Buffer MLA	30 mL	60 mL
Buffer BW1*	22 mL	44 mL
Elution Buffer	10 mL	50 mL

Storage

This kit can be shipped and stored at ambient temperature. Upon receipt, store Lysozyme, Proteinase K and Magnetic Microspheres at 2–8 °C; all other reagents are kept at room temperature. The shelf life is 18 months.

Instructions

Preparations

- 75% Ethanol
- Preparation of Lysozyme solution (50 mg/mL): Add an appropriate volume of Protease Dissolve Buffer to lysozyme powder to reach a final concentration of 50 mg/mL. Invert gently to fully dissolve the powder. Store at −20 °C.
- Preparation of Proteinase K solution (20 mg/mL): Add an appropriate volume of Protease Dissolve Buffer to Proteinase K powder to reach a final concentration of 20 mg/mL. Invert gently to fully dissolve the powder. Store at −20 °C.
- Prior to first use, dilute Buffer BW1 with absolute ethanol as indicated on the bottle label.
- Vigorously shake Magnetic Microspheres for 1–2 minutes before initial use to fully disperse magnetic beads.

Sample Lysis and Digestion

1. Pre-treat samples with lysozyme:

a. Fermentation broth/culture medium (Gram-negative bacteria):
Transfer 1.0–1.5 mL bacterial culture or fermentation broth (containing less than 2×10^9 bacterial cells) into a 1.5 mL centrifuge tube. Centrifuge at 10,000 × g for 1 min to pellet bacteria and discard the supernatant. Add 200 µL Buffer STE, 20 µL Buffer SDS and 10 µL Proteinase K to the pellet, mix thoroughly, and incubate at 65 °C for 30 min. Centrifuge at 13,000 × g for 3 min, transfer 200 µL supernatant and proceed to the subsequent steps..

b. Fermentation broth/culture medium (Gram-positive bacteria):
Transfer 1.0–1.5 mL bacterial culture or fermentation broth (containing less than 1.5×10^9 bacterial cells) into a 1.5 mL centrifuge tube. Centrifuge at 10,000 × g for 1 min to pellet bacteria and discard the supernatant. Add 250 µL Buffer STE and 30 µL Lysozyme, vortex to fully resuspend the pellet, then incubate with shaking at 800–1200 rpm and 37 °C for 30–120 min.

c. Tissue samples:
Weigh 30–50 mg tissue, add 0.5 mL Buffer STE and homogenize thoroughly. Transfer 0.25 mL homogenate, add 30 µL Lysozyme, and incubate with shaking at 800–1200 rpm and 37 °C for 30–120 min.

d. Whole blood samples:

Transfer 1 mL anticoagulated whole blood into a 2 mL centrifuge tube, add 0.5 mL sterile water and 20 µL Buffer SDS, invert 5 times to mix. Centrifuge at 12,000 × g for 3 min to pellet bacteria and remove supernatant. Add 250 µL Buffer STE and 30 µL Lysozyme, then incubate with shaking at 800–1200 rpm and 37 °C for 30–120 min.

e. Secretions, eluates and body fluids:
Take 250 µL of secretions, serum, plasma, hemolytic fluid, swab eluate or other body fluid samples, add 30 µL Lysozyme, and incubate with shaking at 800–1200 rpm and 37 °C for 30–120 min.

f. Dry swab samples:
Place the swab into a 2 mL centrifuge tube, add 300–350 µL Buffer STE and 30 µL Lysozyme, vortex to mix, then incubate with shaking at 800–1200 rpm and 37 °C for 30–120 min.

2. Add 20 µL Buffer SDS and 10 µL Proteinase K, mix well, and incubate with shaking at 65 °C for 30 min.
3. (Optional) Add 10 µL RNase Solution and mix, incubate at ambient temperature for 10 min to digest residual RNA.
4. Centrifuge at 13,000 × g for 3 min, transfer 250–300 µL supernatant and proceed to the subsequent steps..

Experimental Procedure

1. Add 20 µL Magnetic Microspheres and 500 µL Buffer MLA into a 1.5 mL centrifuge tube.
2. Transfer 250–300 µL supernatant obtained from Step 4 into the tube containing Buffer MLA and magnetic beads. Invert 10–15 times to mix thoroughly, incubate at room temperature for 6 min, and vortex several times during incubation. Place the tube on a magnetic rack for 2 min to capture beads, then pour out or aspirate the liquid completely.
3. Add 700 µL Buffer BW1 and vortex for 10 sec. Place on the magnetic rack for 1 min to capture beads, then discard the liquid.
4. Add 700 µL 75% ethanol and vortex for 10 sec. Place on the magnetic rack for 1 min to capture beads, then discard the liquid.
5. Add another 700 µL 75% ethanol and vortex for 10 sec. Place on the magnetic rack for 1 min to capture beads, then discard the liquid.
6. Perform a brief spin-down to collect residual liquid on the tube wall, place the tube back on the magnetic rack, and aspirate all leftover liquid. Air-dry the beads for 10 min.

7. Add 100 μL Elution Buffer and vortex thoroughly to resuspend magnetic beads. Incubate with shaking at 55 °C for 10 min. If a shaking incubator is unavailable, vortex the mixture 2–3 times during incubation to accelerate DNA elution.
8. Place the tube on the magnetic rack for 5 min to capture beads, then transfer the purified DNA supernatant into a new centrifuge tube.