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KitVANCE DURA DNA EXTRACTION FROM G.P. BACTERIA

Designed for isolating high-quality genomic DNA from Gram Positive Bacteria samples

KIT COMPONENTS AND DESCRIPTION

The kit comes in a compact box with labeled vials/bottles. Each kit contains enough reagent for **~50** extractions. Components are divided into consumables (used per extraction) and reusable items. Protocol can be carried out at room temperature.

EQUIPMENT AND REAGENTS SUPPLIED

- L Buffer 1 (10 ml vial)
- L Buffer 2 (5 ml vial)
- Proteinase K (20mg/ml)
- B Buffer (10 mL vial)
- WB1 (25 mL vial)
- WB2 (25 mL vial)
- EB (10 ml)
- Spin Column 1.5 ml (50 unit)
- Collection tubes 1.5 ml (50 units)

PROCEDURE

Total Time: 45 Minutes | Format: Spin Column

1- Transfer 1000 µL of bacterial culture into a microcentrifuge tube. Centrifuge at 10,000 RPM for 2 minutes, then wash the pellet by 70 µL PBS until the solution is clear.
2- Enzyme Step: Add 180 µL of L Buffer 1 and 2mg/ml Lysozyme to the tube.
Incubate at 37°C for 30 minutes, Mix by vertexing for 5 seconds.
3- Add 20 µL Proteinase K and 20 µL of L Buffer 2. Mix by vertexing for 5 seconds. Incubate at 56°C for 10 minutes.

Pro-Tip: The solution should become clear. If it is still cloudy, vortex for 10 more seconds to finish lysis.

4- Add 250 µL Universal Binding Buffer and 200 µL Ethanol (96–100%) or **Isopropanol**. Vortex for 5 seconds.
5- Transfer the entire mixture (~700 µL) into the Silica Spin Column. Centrifuge at 10,000 RPM for 1 minute. Discard the flow-through.
6- Add 500 µL Wash Buffer 1. Centrifuge at 10,000 RPM for 1 minute. Discard the flow-through.

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7- Add 500 µL Wash Buffer 2. Centrifuge at 10,000 RPM for 1 minute. Discard the flow-through.
8- Place the column back into the collection tube. Centrifuge at Max Speed for 1 minutes.

⚠ Critical: This step removes residual ethanol. Do not skip, or your DNA may fail in PCR!

9- Transfer: Place the dry column into a new 1.5 mL tube (provided).
10- Add ~25 µL Elution Buffer directly to the center of the column membrane.
11- For higher yield, use Elution Buffer pre-warmed to 70°C.
12- Centrifuge at 10,000 RPM for 1 minute.

Storage: The purified DNA is now ready for use. Store at 4°C for immediate use or -20°C for long-term storage.

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