DATA SHEET

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Bacteria DNA Preparation Kit

Solution based genomic DNA purification from bacteria

Cat. Nº.	Amount
☐ DPK-113S	100 preparations
☐ DPK-113L	300 preparations

Shipping:
Shipped at ambient temperature

Storage Conditions:
Store at ambient temperature (except RNAse and Proteinase K - store at - 20 ºC)

Shelf life:
12 months

For *in vitro* use only

Kit Contents:

- Cell Resuspension Solution
- Lysozyme (before use, solve in double distilled water to obtain a final concentration of 100 mg/mL) - store at -20 °C
- Cell Lysis Solution
- RNase A (before use, solve in double distilled water to obtain a final concentration of 4 mg/mL) - store at -20 °C
- Protein Precipitation Solution
- Washing Buffer (before use, add 96-99 % Ethanol as indicated on the bottle)
- DNA Hydration Solution

Additional Materials Required:

- Isopropanol (2-propanol) >99%
- Ethanol 96-99%
- 1.5 or 2.0 mL microtub
- Heating Block or Water Bath at 37 °C and 65 °C

Description:
Bacteria DNA Preparation Kit is designed for convenient and fast isolation of genomic DNA from gram-positive and gram-negative bacteria samples. The solution based system minimizes DNA fragmentation that may be problematic in spin-column / filtration based methods. Because phenol or chloroform is not used it is safe and does not produce any harmful waste. Solution based genomic DNA purification kits guarantee minimal DNA fragmentation and yield DNA sized up to 150 kb.

Expected yield:
Yields of genomic DNA will vary from sample to sample depending on the amount, quality and type of material processed. An amount of approx. 40 µg purified DNA per preparation can be expected.

Preparation procedure:
Before start, provide >99 % Isopropanol (2-propanol) (not included in the kit).

For S pack (100 preps): Add 250 µL dd-water to the Lysozyme tube, 200 µL dd-water to the RNase A tube and 44 mL 96-99 % Ethanol (not included in the kit) to the Washing Buffer bottle.

For L pack (300 preps): Add 250 µL dd-water to each Lysozyme tube, 200 µL dd-water to each RNase A tube and 132 mL 96-99 % Ethanol (not included in the kit) to the Washing Buffer bottle.

Buffer	DPK-113S 100 preps	DPK-113L 300 preps
Cell Ressuspension solution	30 mL	90 mL
Lysozyme (100 mg/ml)	25 mg	3 x 25 mg
Cell Lysis Solution	30 mL	90 mL
Rnase A (4 mg/ml)	0.8 mg	3 x 0.8 mg
Protein Precipitation Solution	10 mL	30 mL
Washing buffer	add 44 mL EtOH (final vol. 55 mL)	add 132 mL EtOH (final vol. 165 mL)
DNA Hydration Solution	10 mL	30 mL

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1a. Cell Lysis for Gram-Positive bacteria:

- Transfer 1 mL of cultured cells into a 1.5 mL microtube
- To harvest the cells centrifuge at 15,000 g for 1 min and discard the supernatant.
- Resuspend the cell pellet in 300 µL of Cell Resuspension Solution.
- Add 2 µL of Lysozyme Solution and mix well by inverting.
- Incubate the tube at 37 °C for 60 min with occasional inverting.
- Centrifuge at 15,000 g for 1 min and discard the supernatant.
- Resuspend the pellet in 300 µL of Cell Lysis Solution.

1b Cell Lysis for Gram-Negative Bacteria:

- Transfer 1 mL of cultured cells into a 1.5 mL microtube.
- To harvest the cells centrifuge at 15,000 g for 1 min and discard the supernatant.
- Resuspend the pellet in 300 µL of Cell Lysis Solution.

2 RNase Treatment:

- Add 1.5 µL of RNase A Solution and mix by inverting.
- Incubate at 37 °C for 15-30 min and cool on ice for 1 min.

3 Protein Precipitation:

- Add 100 µL of Protein Precipitation Solution and vortex vigorously for 20-30 sec.
- Centrifuge at 15,000 g for 5 min.

4 DNA Precipitation:

- Transfer the supernatant to a clean 1.5 mL microtube containing 300 µL Isopropanol >99 %.
- Mix the sample by inverting gently for 1 min.
- Centrifuge at 15,000 g for 1 min (DNA should be visible as a small white pellet).
- Discard the supernatant and drain tube briefly on clean absorbent paper.
- Add 500 µL Washing Buffer and invert the tube several times to wash the DNA pellet.
- Centrifuge at 15,000 g for 1 min.
- Discard the ethanol carefully.
- Air dry at room temperature for 10-15 min.

5 DNA Hydration:

- Add 50-100 µL of DNA Hydration Solution to the dried DNA pellet.
- Hydrate the DNA by incubating at 65 °C for 60 min.
- Store the DNA at 4 °C. For long time storage, store the sample at -20 °C or -80 °C.