

Bacteria DNA Preparation Kit

Genomic DNA purification from bacteria

DNA Preparation Kit

Cat.-No.	Amount
PP-206S	100 preparations

For *in vitro* use only
Quality guaranteed for 12 months
Store at room temperature
For long term storage place Lysozyme and RNase A lyophilisates at -20°C
Lysozyme and RNase A Solutions should be stored at -20°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.

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.

Kit contents

Cell Resuspension Solution
Lysozyme (before use, solve in 250 µl water to a final concentration of 100 mg/ml)
Cell Lysis Solution
RNase A (before use, solve in 200 µl water to a final concentration of 4 mg/ml)
Protein Precipitation Solution
DNA Hydration Solution

To be provided by you

Isopropanol (2-propanol) >99%
Ethanol 80%
Microtubes 1.5 ml

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Preparation procedure

Before start, provide >99% Isopropanol (2-propanol) and 80% Ethanol (both not included in the kit).

Add 250 µl dd-water to the *Lysozyme* and 200 µl dd-water to the *RNase A lyophilisate*. The *Lysozyme* and *RNase A Solutions* should be stored at -20°C.

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 *Ethanol 80%* 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.