Manual

Bacteria DNA Preparation Kit

Genomic DNA purification from bacteria

DNA Preparation Kit

Cat.-No.	Amount
PP-206	400 preparations

For *in vitro* use only
Quality guaranteed for 12 months
Store at room temperature

Kit contents

Cell Resuspension Solution

Lysozyme (before use solve in water to a final concentration of 100 mg/ml)

RNase A (before use solve in 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

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 other spin-column/filtration based method. Because phenol or chloroform is not used it is safe and does not produce any harmful waste.

Preparation procedure

Before start, provide >99% Isopropanol (2-propanol) and 80% Ethanol (both not included in the kit).
Solve the *Lysozyme* and *RNase lyophilisates* in dd-water as indicated on each bottle. The *Lysozyme Solution* and *RNase A Solution* should be stored at 4°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.
- Place 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 15-60 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*

- Pour the supernatant into a clean 1.5 ml microtube containing 300 µl *Isopropanol >99%*.
- Mix the sample by inverting gently 50 times.
- Centrifuge at 15,000 g for 1 min. (DNA should be visible as a small white pellet.)
- Pour off 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.
- Pour off the ethanol carefully and wash again with 500 µl of *Ethanol 80%*.
- Invert and drain the tube on clean absorbent paper and allow to air dry for 10-15 min.

5. *DNA Hydration*

- Add 100 µl of *DNA Hydration Solution* to the dried DNA pellet.
- Hydrate the DNA by incubating for 1 h at 65°C.
- Store the DNA at 4°C. For long time storage, store the sample at -20°C or -80°C.