

Bacteria DNA Preparation Kit

DNA purification from bacteria

Solution based genomic DNA Preparation Kit

	Cat.-No.	Amount
	PP-206XS	20 preparations
	PP-206S	100 preparations
	PP-206L	500 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.

Kit contents

Cell Resuspension Solution

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

Cell Lysis Solution

RNase A (before use, solve in double distilled water to obtain a final concentration of 4 mg/ml)

Protein Precipitation Solution

Washing Buffer (before use, add 96-99% Ethanol as indicated on the bottle)

DNA Hydration Solution

To be provided by you

Isopropanol (2-propanol) >99%

96-99% Ethanol

Microtubes 1.5 ml

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.

Buffer	PP-206XS	PP-206S	PP-206L
	20 preps	100 preps	500 preps
Cell Resuspension Solution	6.4 ml	32 ml	160 ml
Lysozyme	5 mg (before use, solve in 50 µl dd-water to obtain a final concentration of 100 mg/ml)	25 mg (before use, solve in 250 µl dd-water to obtain a final concentration of 100 mg/ml)	5 x 25 mg (before use, solve each vial in 250 µl dd-water to obtain a final concentration of 100 mg/ml)
Cell Lysis Solution	6.4 ml	32 ml	160 ml
RNase A	0.16 mg (before use, solve in 40 µl dd-water to obtain a final concentration of 4 mg/ml)	0.8 mg (before use, solve in 200 µl dd-water to obtain a final concentration of 4 mg/ml)	5 x 0.8 mg (before use, solve each vial in 200 µl dd-water to obtain a final concentration of 4 mg/ml)
Protein Precipitation Solution	2.2 ml	11 ml	55 ml
Washing Buffer	add 9.6 ml Ethanol (final volume 12 ml)	add 48 ml Ethanol (final volume 60 ml)	add 120 ml Ethanol to each bottle (final volume 150 ml each)
DNA Hydration Solution	2.2 ml	11 ml	55 ml

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Preparation procedure

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

For XS pack (20 preps): Add 50 µl dd-water to the *Lysozyme* tube, 40 µl dd-water to the *RNase A* tube and 9.6 ml 96-99% Ethanol (not included in the kit) to the *Washing Buffer* bottle.

For S pack (100 preps): Add 250 µl dd-water to the *Lysozyme* tube, 200 µl dd-water to the *RNase A* tube and 48 ml 96-99% Ethanol (not included in the kit) to the *Washing Buffer* bottle.

For L pack (500 preps): Add 250 µl dd-water to each *Lysozyme* tube, 200 µl dd-water to each *RNase A* tube and 120 ml 96-99% Ethanol (not included in the kit) to each *Washing Buffer* bottle.

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 *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.