DATA SHEET

Jena Bioscience

Plasmid Mini-Prep Kit - Column Kit

Column based isolation of plasmid DNA

Cat. No.	Amount
PP-204S	50 preparations
PP-204L	250 preparations

For general laboratory use.

Shipping: shipped at ambient temperature

Storage Conditions: store at ambient temperature (except RNase - store at -20 °C)

Shelf Life: 12 months

Description:
Fast-n-Easy Plasmid Mini-Prep Kit is designed for isolation of high-purity plasmid or cosmid DNA from bacterial cells for subsequent amplification, sequencing, restriction digests or transformations. The 2-step alkaline lysis procedure and binding column based preparation provide a fast, easy and efficient way of DNA isolation without shearing or significant loss of product. It allows elution in a small volume of low-salt buffer. Time-consuming phenol-chloroform extraction or alcohol precipitation is not required.
The Lysis Buffer contains an integrated pH indicator to easily control the optimal pH value for DNA binding. Efficient DNA binding (for Column loading) requires a pH of 7.5 that is indicated by a color change of the indicator to bright yellow.
The kit can either be used in micro-centrifuges or on vacuum manifolds. It enables the extraction of plasmid DNA up to 10 kb length and yields up to 20 µg DNA per preparation.
The eluted high-quality plasmid DNA is ready to use for a variety of down-stream application. For subsequent *in vitro* translation we recommend to add RNase Inhibitor (Cat.-No. PCR-504) or the application of an additional spin-column or phenol-chloroform based purification step. This avoids any risk of carry-over contamination with RNase due to the previous neutralization step.

Content:

- Lysis Buffer including pH indicator
- Neutralization Buffer (before use, add RNase A and store at 4°C)
- RNase A - store at -20°C
- Activation Buffer
- Washing Buffer (before use, add 96-99% Ethanol as indicated on the bottle)
- Elution Buffer
- Binding Columns
- 2 ml Collection Tubes

Additional Materials Required:

- 96-99% Ethanol
- 1.5 ml microtubes

Preparation Procedure:
The DNA purification follows a simple binding, washing, and eluting procedure. The optional secondary washing step minimizes the salt content of the purification product. Before starting, add the following components to the respective bottles:

- Add RNase A as indicated in the table below to the Neutralization Buffer and mix well. Neutralization Buffer containing RNase A should be stored at 4 °C.
- The activity of dissolved RNase A in Neutralization Buffer may decrease after several months and small amounts of RNA may be co-purified. In case RNA is detected after plasmid purification, add the additional RNase A to the Neutralization Buffer in order to retrieve enzyme activity.

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- Add 96-99 % Ethanol (not included in the kit) to the Washing Buffer as indicated on the bottle. Please note that the Ethanol concentration of Washing Buffer may decrease during long term storage resulting in a drop-down of the final DNA yield.

Buffer	**PP-204S** 50 preps	**PP-204L** 250 preps
Lysis Buffer	16 ml	80 ml
RNase A	2x 4 mg	10x 4 mg
Neutralization Buffer	16 ml, add 1x 4 mg RNase A	80 ml, add 5x 4 mg RNase A
Activation Buffer	6 ml	30 ml
Washing Buffer	add 64 ml Ethanol (final volume 80 ml)	add 160 ml Ethanol to each bottle (final volume 200 ml each)
Elution Buffer	5 ml	25 ml

1 Cell Harvest and Lysis:

- Harvest the bacterial cell culture (1-3 ml) by centrifugation.
- Resuspend pelleted bacterial cells in 300 µl Lysis Buffer by pipetting or vortexing for 1 min.

2 Neutralization:

- Add 300 µl of Neutralization Buffer (containing RNase A) to sample and mix gently by inverting the tube 4-6 times (do not vortex!).
- Centrifuge at 10,000 g for 5 min at room temperature in a micro-centrifuge.
- The color of the binding mixture should change to bright yellow indicating a pH of 7.5 required for optimal DNA binding. An orange or violet color shows a pH >7.5 and indicates an inefficient DNA adsorption. In this case, it is recommended to adjust the pH of the mixture by addition of a small volume of 3 M sodium acetate, pH 5.0 before proceeding.

3 Column Activation:

- Place a Binding Column into a 2 ml collection tube.
- Add 100 µl of Activation Buffer into the Binding Column.
- Centrifuge at 10,000 g for 30 sec in a micro-centrifuge.

4 Column Loading:

- Apply the supernatant from step 2 into the activated Binding column by decanting or pipetting.
- Centrifuge at 10,000 g for 30 sec.
- Discard the flow-through.

5 Column Washing:

- Place the DNA loaded Binding Colum into the used 2 ml tube.
- Apply 500 µl of Washing Buffer (containing Ethanol) to the Binding Column.
- Centrifuge at 10,000 g for 30 sec and discard the flow-through.

Optional Secondary Washing: Recommended only for DNA >200 bp, if highly purified DNA (for DNA sequencing, transfection etc.) is required.

- Add 700 µl of Washing Buffer to the Binding Column.
- Centrifuge at 10,000 g for 30 sec and discard the flow-through.
- Centrifuge again for 2 min to remove residual Washing Buffer.

6 Elution:

- Place the Binding Column into a clean 1.5 ml microtube (not provided in the kit).
- Add 30-50 µl Elution Buffer or dd-water to the center of the column membrane.
- Incubate for 1 min at room temperature.
- Centrifuge at 10,000 g for 1 min to elute DNA.

Ultra-pure plasmid DNA is now ready to use.