Product Information Sheet and Protocol

Product Name: Plasmid Mini-Prep Kit*
Item No.:

Item Number	Preparations
SS-PMK-10	10
PMK-50	50
PMK-250	250

Storage and Handling:
Store at room temperature upon arrival (Store RNAse at -20°C).

Product Description:
Empirical Bioscience 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 are 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 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 applications. For subsequent *in vitro* translation we recommend adding RNase Inhibitor 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.

Kit contents:
Lysis Buffer, Neutralization Buffer (before use, add Rnase A and store at 4°C), Activation Buffer, Washing Buffer (add 96-99% Ethanol as indicated), Elution Buffer, Rnase A, and Spin Columns & 2 ml Collection Tubes.

Additional Materials not provided:
96-99% Ethanol
1.5mL microtubes

Preparation Procedure:
Empirical Bioscience Mini-Prep 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 some Neutralization Buffer to the tube of lyophilized Rnase A. Mix well to resuspend and then add the resuspended RNase A of one vial 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 RNAs are detected after plasmid purification, add the additional RNase A to the Neutralization Buffer in order to enhance enzyme activities.
- 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 potential drop of the final DNA yield.

Buffer	SS-PMK-10	PMK-50	PMK-250
Lysis Buffer	3.2mL	16mL	80mL
Neutralization Buffer	3.2mL, add 0.8mg RNase A	16mL, add 1x4mg RNase A	80mL, add 5x4mg RNase A
Activation Buffer	1.2mL	6mL	30mL
Washing Buffer	Add 12mL Ethanol (15mL final Volume)	Add 64mL Ethanol (80mL final Volume)	Add 160mL Ethanol (final Volume of 200mL)
Elution Buffer	1mL	5mL	25mL
RNase A	1 x 0.8mg	2 x 4mg	10 x 4mg

Protocol:

Cell Harvest and Lysis:

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

Neutralization:

- Add 300µl of Neutralization Buffer (containing RNase A) to sample and mix gently by inverting the tube 4-6 times (do not vortex).
    - 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 with pH >7.5 indicates there will be 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.
- Centrifuge at 10,000 x g for 5 min at room temperature in a micro-centrifuge.

Column Activation:

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

Column Loading:

- Apply the supernatant from Neutralization into the activated Binding Column by decanting or pipetting.
- Centrfuge at 10,000 x g for 30 sec.
- Discard the flow-through.

Column Washing:

- Place the DNA loaded Binding Column into the used 2mL tube.
- Apply 500µl of Washing Buffer (containing Ethanol) to the Binding Column.
- Centrifuge at 10,000 x g for 30 sec and discard the flow-through.
    - **Optional Secondary Washing:** Recommended only for DNA > 200 bp, if highly purified DNA is required.
    - Add 700µl of Washing Buffer to the Binding Column.
    - Centrifuge at 10,000 x g for 30 sec and discard the flow-through.
- Centrifuge again for 2 min to remove residual Washing Buffer.

Elution:

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