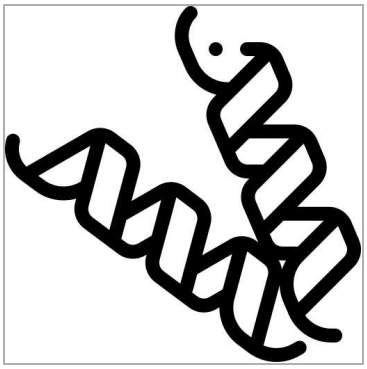

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OPEN ACCESS

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Protocol status: Working
We use this protocol and it's working

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Fast-n-Easy Plasmid Mini-Prep Kit (cellco)

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DISCLAIMER

This protocol is from the DATA SHEET of the following kit of cellco:

Fast-n-Easy Plasmid Mini-Prep Kit
Column based isolation of plasmid DNA

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ABSTRACT

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-consumingphenol-chloroformextractionoralcohol precipitation is not required.

ATTACHMENTS

DPK_104_en.pdf

GUIDELINES

For research use only!

MATERIALS

Kit Contents:

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

BEFORE START INSTRUCTIONS

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 lower than 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 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.
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:
ŸAdd the RNase A to the Neutralization Buffer and mix well. Neutralization Buffer containing RNase A should be stored at 4 °C.
ŸThe 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 additional RNase A to the Neutralization Buffer in order to enhance enzyme activities.
ŸAdd 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.

1 5m

- Harvest the 1 ~ 3 mL of bacterial cell culture by 10000 rpm, 00:05:00 .
- Discard the supernatant media.
- Resuspend of cell pellet with remained media by vigorously vortexing or pipetting.
- Add 300 µL of Buffer S1. (**Buffer S1** is Cell lysis buffer).
- **Inverting** the tube immediately **2 ~ 3 times**.
- **Do Not Vortexing and Pipetting.**

Note

At this time, cell ressuspension with Buffer S1(Lysis Buffer) by vorteing or pipetting can clivage gDNA. The cleaved gDNA affects the plasmid DNA preparation step and can be isolated together with the plasmid DNA. This reduces the efficiency of plasmid DNA prep. For this reason, mix only by the inverting method after adding Buffer S1.

2 Neutralization: 5m

- 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 10000 x g, Room temperature, 00:05:00 in a microcentrifuge.

Note

NOTE: The color of the binding mixture should change to bright yellow indicating a pH lower than 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: 30s

- Place a Binding Column into a 2 ml collection tube.
- Add 100 µL of **Activation Buffer** into the **Binding Column**.
- Centrifuge at 10000 x g, 00:00:30 in a micro-centrifuge.

4 Column Loading: 30s

- Apply the supernatant from **step 2** into the activated **Binding Column** by decanting or pipetting.
- Centrifuge at 10000 x g, 00:00:30.
- Discard the flow-through from the collection tube.

5 Column Washing: 30s

- Apply 500 µL of Washing Buffer (**containing Ethanol**) to the Binding Column.
- Centrifuge at 10000 x g, 00:00:30 and discard the flow-through.

5.1 Optional Secondary Washing:Recommended only for DNA >200 bp, if highly purified DNA (for DNA sequencing, transfectionetc.) is required. 2m 30s

- Add 700 µL of Washing Buffer to the Binding Column.
- Centrifuge at 10000 x g, 00:00:30 and discard the flow-through.
- Centrifuge again for 10000 x g, 00:02:00 to **remove residual Washing Buffer.**

6 Elution 2m

- 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 00:01:00 at Room temperature.
- Centrifuge at 10000 x g, 00:01:00 to elute DNA.