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Kit for for Isolation of plasmid DNA from bacterial cells

Cat. No. Plasmid-50-mini, Plasmid-250-mini

Important!

We are constantly improving the protocol for the kit. Please use the protocol provided with the product.
Updated March 2022

Description

The kit is designed for isolation and purification of plasmid DNA from bacterial cells cultures. The protocol consists of two main operational stages: alkaline lysis of bacterial cells and subsequent binding of nucleic acids from a lysed sample on a silica-gel membrane, followed by washing and the elution of the purified sample.

The isolated DNA can be used for PCR, restriction, sequencing, transformation, transfection, and other applications.

Kit Components

	Plasmid-50 mini 50 preps	Plasmid-250 mini 250 preps
Suspension buffer SB	15 ml	70 ml
Lysis buffer LB	15ml	2x35 ml
Neutralization buffer NB	24 ml	110ml
Wash buffer WB1 (concentrate)	22.5 ml	2x52.5 ml
Wash buffer WB2 (concentrate)	6 ml	2x14 ml
Elution buffer EB	2x5 ml	50 ml
Collection tubes and spin columns	50 pcs	250 pcs

Safety Precautions

Caution! Lysis buffer LB, Neutralization buffer NB, Wash buffer WB1 contain irritant and toxic chemicals. When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. Buffers are toxic in contact with skin and insides, causing burns.

In case of a contact with skin, wash immediately with plenty of water and detergents. Visit a doctor if necessary.

Operation

Fast LB components are stable after opening the vial at temperatures from 15°C to 25°C during the entire shelf life, assuming that the vials are sufficiently sealed. Proteinase K solution is stable after opening for 12 months. The mixture of lysis buffer LB and proteinase K can be stored for 14 days at a temperature not exceeding 2-8°C.

Operation conditions
Ambient temperature: 15 – 25 °C;
Relative air humidity: No more than 80 %;
Atmospheric pressure: 630 – 800 mm Hg

Equipment and Reagents to be Supplied by User

- Microcentrifuge with rotor for 1.5 ml tubes, speed 10000 rcf
- 1.5 ml microcentrifuge tubes
- Ethanol, 96-100%
- RNase A solution, 10 mg/ml

Before starting the procedures:

1) Wash buffer WB1. Add ethanol (95-99 %) to WB1 buffer and mix.
   - 1 prep. To obtain 500 µl of WB1 buffer, add 125 µl of ethanol to 375 µl of WB1 buffer (concentrate).
   - 50 preps. To obtain 30 ml of WB1 buffer, add 7.5 ml of ethanol to 22.5 ml of WB1 buffer (concentrate).
   - 250 preps. To obtain 70 ml of WB1 buffer, add 17.5 ml of ethanol to 52.5 ml of WB1 buffer (concentrate).
2) Wash buffer WB2. Add ethanol (95-99 %) to WB2 buffer and mix.
   - 1 prep. To obtain 500 µl of WB2 buffer, add 400 µl of ethanol to 100 µl of WB2 buffer (concentrate).
   - 50 preps. To obtain 30 ml of WB2 buffer, add 24 ml of ethanol to 6 ml of WB2 buffer (concentrate).
   - 250 preps. To obtain 70 ml of WB2 buffer, add 56 ml of ethanol to 14 ml of WB2 buffer (concentrate).

Note! It is recommended to add ethanol to the aliquots of the WB1 and WB2 buffers, since ethanol may partially evaporate when storing the buffer for several months.

Plasmid DNA isolation protocol.

Use 1-5 ml of bacterial cell suspension (the amount depends on the copy number and length of the plasmid) for plasmid DNA isolation. DNA isolation is carried out at room temperature (15-25 °C).

Sample lysis and column loading

1) Precipitate bacterial cells from the culture medium by centrifugation at 10.000 rcf for 1 min.
2) Add 250 µl of SB buffer to the cell pellet. Suspend by pipetting.
3) Add 2 µl of RNase A solution (10 mg/ml) and 250 µl of LB buffer to the cell suspension. Mix gently by hand turning the tube upside-down 5-10 times until a homogeneous mixture is obtained. Do not use a vortex!

Note: RNase A is not included in the kit.

4) Add 400 µl of NB neutralization buffer. Mix gently by hand turning the tube upside-down 5-10 times until a homogeneous mixture is obtained. Do not use a vortex!

Note: Mix the suspension immediately after adding NB buffer to avoid the formation of coarse particles.

5) Centrifuge at 10.000 rcf for 10 min.

6) Apply 800 µl of supernatant liquid to the column. Centrifuge at 10.000 rcf for 30 sec. Discard the flow-through.

Column washing

1) Add 500 µl of WB1 buffer to the column. Centrifuge at 10.000 rcf for 30s. Discard the flow-through.

Note: *Ensure that ethanol was added to the WB1 buffer.*

2) Add 500 µl of WB2 buffer wash buffer to the column. Centrifuge at 10.000 rcf for 30s. Discard the flow-through.

Note: *Ensure that ethanol was added to the WB2 buffer.*

3) Centrifuge at 10.000 rcf for 3 min to completely remove the WB2 buffer.

DNA elution

1) Transfer the column into a new 1.5 ml microcentrifuge tube (not included in the kit).

2) Carefully apply 60-100 µl of EB buffer directly to the center of the column membrane. Incubate for 3 min at room temperature (15-25 °C). Centrifuge at 10.000 rcf for 1 min.

Note: The EB buffer contains 10 mM Tris • HCl (pH 8.0). The sample can also be eluted with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0-8.5) or water (pH 8.0-8.5). EDTA may inhibit following enzymatic reactions.

3) Store the eluate containing DNA at -20 °C. For long-term storage, it is recommended to add EDTA (pH 8) to its final concentration of 0.1-1 mM.

Analysis of isolated DNA.

DNA can be analyzed can be analyzed by gel electrophoresis in 1% agarose gel.

The amount of isolated DNA can be estimated by using UV spectrometry.
The maximum of absorption for DNA corresponds to $\lambda = 260$ nm.
DNA concentration (µg/ml) can be calculated using the following formula:
A_{260} * dilution * 50 µg/ml.
Typical optical density ratios are A_{260}/A_{280} ~1.7-2.0.

Storage

All components of the kit can be stored at room temperature (15-25 °C) for up to 12 months.

Important! Close bottle containing LB buffer immediately after use to avoid acidification via exposure of CO_2 from the air.

Shipping

All components of the kit are shipped at room temperature.