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Column plasmid DNA isolation mini kit (Plasmid-mini)

Cat. No. Plasmid-10-mini, Plasmid-50-mini, Plasmid-250-mini

Important!

We are regularly improving the protocol for working with the kit. Please use the protocol included with the kit. The latest version of the protocol is available on the website of Biolabmix LLC (www.biolabmix.ru).
The kit is intended for research purposes only.
The protocol was updated on May 2024.

Description

The kit is designed for isolation and purification of plasmid DNA from *E. coli* bacterial cell cultures. It is optimized for isolation from up to 5 ml of cell suspension (depending on plasmid copy number).
The protocol consists of two main operational stages: alkaline lysis of bacterial cells and subsequent binding of plasmid DNA from a lysed sample on a silica-gel membrane of the column, followed by washing and elution of the purified sample. Column capacity is up to 30 μg. Lysis buffer contains a blue pH indicator to provide better control of conditions on the neutralization step. During the neutralization, the solution becomes colorless.
The isolated DNA can be used for PCR, restriction, sequencing, transformation, transfection and other genetic engineering applications.

Contents

	Plasmid-10-mini 10 preps	Plasmid-50-mini 50 preps	Plasmid-250-mini 250 preps	
			Var. 1	Var. 2
Suspension buffer SB	3 ml	15 ml	2x50 ml	100 ml
Lysis buffer LB (with pH indicator)	3 ml	15 ml	5x15 ml	5x15 ml
Neutralization buffer NB	3 ml	15 ml	2x50 ml	100 ml
Wash buffer WB1	5.5 ml	30 ml	3x50 ml	2x75 ml
Wash buffer WB2 (concentrate)	1.1 ml	6 ml	3x10 ml	2x15 ml
Elution buffer EB	5 ml	15 ml	60 ml	60 ml
RNAse A solution, 10 mg/ml	25 μl	115 μl	550 μl	550 μl
Collection tubes and spin columns	10 pcs	50 pcs	250 pcs	250 pcs

The Plasmid-250-mini kit is supplied in one of two package variants.

Safety information

Caution! Lysis LB, neutralization NB and wash WB1 buffers contain reagents, which are irritating and toxic if they come in contact with skin or inside, causing burns. When working, always wear a suitable lab coat, disposable gloves, and protective goggles.
Caution! Wash buffer WB1 contain isopropanol, which is irritating and toxic. Do not work with these solutions near open flames.
In case of a contact with skin, wash immediately with plenty of water and detergents. Visit a doctor if necessary.

Operation

Components: SB, LB, NB, WB1, WB2, EB and RNAse A solution are stable after opening throughout the entire shelf life if stored in appropriate conditions and sufficiently sealed. Storage conditions are indicated on the kit and reagents labels.

Operation conditions

Ambient temperature from +15 to +25 °C;
Relative air humidity less than 80 %;
Atmosphere pressure 630 – 800 mmHg.

Equipment and reagents to be supplied by user

- Microcentrifuge for 1.5-2 ml tubes, speed 12000 rcf;
- Single-channel variable volume micropipettes with disposable tips;
- Disposable gloves;
- 1.5 ml microcentrifuge tubes;
- Ethanol, 96 – 100%.

Before starting the procedures

Prepare wash buffer WB2

- 1 prep, 500 µl WB2. Add 400 µl of ethanol (96-100%) to 100 µl of WB2 (concentrate).
- 10 preps. Add 4.4 ml of ethanol (96-100%) to 1.1 ml of WB2 (concentrate).
- 50 preps. Add 24 ml of ethanol (96-100%) to 6 ml of WB2 (concentrate).
- 250 preps. Var. 1. Add 40 ml of ethanol (96-100%) to 10 ml of WB2 (concentrate).
- 250 preps. Var. 2. Add 60 ml of ethanol (96-100%) to 15 ml of WB2 (concentrate).

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

DNA isolation protocol

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

1) Preparing and lysing the samples

1. Precipitate bacterial cells from the culture medium by centrifugation at 12000 rcf for 1 min or use the method accepted in your laboratory. Remove the supernatant.
2. Add 250 µl SB to the pellet. Resuspend by pipetting.
3. Add 2 µl of RNAse A solution (10 mg/ml) and 250 µl LB. Mix carefully by inverting the sealed microtube 5 – 10 times until mixture becomes homogeneous. Do not use vortex!

Important! Close the bottle containing LB tightly immediately after use to prevent acidification from CO_2 in the air.

Note: buffer LB contains blue pH-indicator.

4. Incubate the mixture not more than 3 min.
5. Add 250 µl NB. Mix carefully by inverting the sealed microtube 5 – 10 times until mixture becomes colorless. Do not use vortex!

Note: mix the suspension immediately after adding NB to prevent forming of large particles. Continue mixing until all the blue particles become colorless.

6. Incubate for 5 min.
7. Centrifuge for 15 min, 12000 rcf.

2) Column loading

Transfer supernatant to the column. Centrifuge for 30 s, 12000 rcf. Discard the flow-through.

Note: if some particles didn't precipitate during centrifugation, carefully transfer the supernatant to the column avoiding capture precipitate.

3) Column wash

1. Add 500 µl WB1 to the column. Centrifuge for 30 s, 12000 rcf. Discard the flow-through.
2. Add 500 µl WB2 to the column. Centrifuge for 30 s, 12000 rcf. Discard the flow-through.

Note: ensure that ethanol was added to the WB2 buffer.

3. Centrifuge column for 3 min, 12000 rcf to completely remove the WB2.

4) DNA elution

1. Transfer the column into a new 1.5 ml microcentrifuge tube (not included).
2. Carefully apply 60-200 µl EB directly to the center of the column membrane. Incubate for 3 min at room temperature (15-25 °C). Centrifuge for 1 min, 10000 rcf.

- Increasing the elution volume leads to higher DNA yields and lower DNA concentration.

- Repeating the elution step with new aliquot of EB or reloading the eluted sample to the column allows to increase DNA yields.
- Elution buffer EB is 0.01 M Tris•HCl (pH 8.0). The sample can also be eluted with TE buffer (0.01 M Tris•HCl, 0.001 M EDTA, pH 8.0 – 8.5) or with water (pH 8.0 – 8.5, adjust pH by NaOH solution).

3. Store the eluate containing DNA at -20 °C.

Optional. For long-term storage it is recommended to add EDTA (pH 8) to the final concentration of 0.1-1 mM. EDTA can inhibit enzymatic reactions, for example, PCR.

DNA analysis

DNA can be analyzed by gel electrophoresis in 1% agarose gel.
The amount of isolated DNA can be estimated using UV spectrometry.
The maximum of absorption for DNA corresponds to λ = 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.

Additional ordering Information

- Buffers for agarose gel electrophoresis: TAE (Cat. No. BE-DNA-500, BE-DNA-1000), TBE (Cat. No. TBE-500).
- Ethidium bromide (Cat. No. EtBr-10) for nucleic acid visualisation gel electrophoresis.
- Nucleic acids loading buffers (Cat. No. D-3001, D-3002, D-3003) for agarose gel electrophoresis.
- DNA ladders (Cat. No. S-8000, S-8100, S-8103, S-8055, S-8250, S-8150).

Storage

The kit can be stored at room temperature (15-25 °C). RNAse A solution should be stored at -18 °C to -24 °C. See expiration date on the package label.

Shipping

All components of the kit are shipped at room temperature (15-25 °C). Allowed shipping for 14 days at a temperature below 25 °C.