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Column plasmid DNA isolation maxi kit (Plasmid-maxi)

Cat. No. Plasmid-20-maxi

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 March 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 100 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 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 1.5 mg. 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.

Important! Plasmid DNA isolation can be carried out both in low-speed centrifuges (3000 rcf) with a bucket or angle rotor (see protocol 1), and in high-speed centrifuges (14000 rcf) with angle rotor (see protocol 2).

Important! Isolated plasmid DNA can be concentrated by precipitation (see protocol 3) using a refrigerating microcentrifuge with speed 12000 rcf at +4 °C.

Contents

	Plasmid-20 maxi 20 preps Var. 1	Plasmid-20 maxi 20 preps Var. 2
Suspension buffer SB	4x50 ml	2x100 ml
Lysis buffer LB (with pH indicator)	4x50 ml	2x100 ml
Neutralization buffer NB	4x50 ml	2x100 ml
Wash buffer WB1	5x50 ml	2x125 ml
Wash buffer WB2 (concentrate)	5x10 ml	2x25 ml
Elution buffer EB	60 ml	60 ml
RNAse A solution, 10 mg/ml	1250 µl	1250 µl
Collection tubes and spin columns	20 pcs	20 pcs
Precipitation solution PS1	5 ml	5 ml
Precipitation solution PS2	2x10 ml	2x10 ml
Wash solution WS (концентрат)	3 ml	3 ml

The Plasmid-20-maxi 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 WB1 and precipitation PS2 buffers 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, RNAse A solution, PS1, PS2 and WS 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

- Centrifuge for 50 ml tubes, bucket or angle rotor, speed 3000 or 14000 rcf;
- 50 ml centrifuge tubes (conical bottom or other fitting tubes);
- Single-channel variable volume micropipettes with disposable tips;
- Disposable gloves;
- 1.5 ml microcentrifuge tubes;
- Ethanol, 96 – 100%.

Optional (protocol 3, precipitation of plasmid DNA)

- Microcentrifuge for 1.5-2 ml tubes, speed 12000 rcf, cooling down to +4 °C.

Before starting the procedures

Prepare wash buffer WB2

- **1 prep, 10 ml WB2.** Add 8 ml of ethanol (96-100%) to 2 ml of WB2 (concentrate).
- **20 preps. Variant 1.** Add 40 ml of ethanol (96-100%) to 10 ml of WB2 (concentrate).
- **20 preps. Variant 2.** Add 100 ml of ethanol (96-100%) to 25 ml of WB2 (concentrate).

Optional. For protocol 3 (precipitation of plasmid DNA) prepare wash solution WS (80% ethanol).

- **1 prep, 500 µl.** Add 400 µl of ethanol (96-100%) to 100 µl of WS (concentrate, water type I).
- **20 preps, 15 ml.** Add 12 ml of ethanol (96-100%) to 3 ml of WS (concentrate, water type I).

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

Protocol 1. Isolation of plasmid DNA using low-speed centrifuge (speed not less than 3000 rcf)

Use 50-100 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 3000 rcf for 15 min or use the method accepted in your laboratory. Remove the supernatant.
2. Add 7.5 ml SB to the pellet. Suspend by pipetting.
3. Add 50 µl of RNAse A solution (10 mg/ml) and 7.5 ml LB. Mix carefully by inverting the sealed tube 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 7.5 ml NB. Mix carefully by inverting the sealed tube 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 60 min, 3000 rcf.

2) Column loading

1. Transfer 12.5 ml of supernatant to the column. Centrifuge for 2 min, 3000 rcf. Discard the flow-through.

Note: if some particles did not precipitate during centrifugation and are floating on the surface, try not to capture them when collecting the supernatant.

2. Load remaining supernatant to the column. Centrifuge for 2 min, 3000 rcf. Discard the flow-through.

3) Column wash

1. Add 10 ml WB1 to the column. Centrifuge for 2 min, 3000 rcf. Discard the flow-through.
2. Add 10 ml WB2 to the column. Centrifuge for 2 min, 3000 rcf. Discard the flow-through.

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

3. Centrifuge column for 5 min, 3000 rcf to completely remove the WB2.

4) DNA elution

1. Transfer the column into a new collecting tube (included in the kit).
2. Apply 1 ml EB directly to the center of the column membrane. Incubate for 3 min at room temperature (15-25 °C). Centrifuge for 3 min, 3000 rcf.

3. Transfer eluate to a new clear 1.5 ml microtube (not included).
Note: 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).
4. 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.

Protocol 2. Isolation of plasmid DNA using high-speed centrifuge (speed not less than 14000 rcf)

Use 50-100 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 7.5 ml SB to the pellet. Suspend by pipetting.
3. Add 50 µl of RNAse A solution (10 mg/ml) and 7.5 ml LB. Mix carefully by inverting the sealed tube 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 7.5 ml NB. Mix carefully by inverting the sealed tube 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 20 min, 14000 rcf.

2) Column loading

1. Transfer 12.5 ml of supernatant to the column. Centrifuge for 1 min, 14000 rcf. Discard the flow-through.

Note: if some particles did not precipitate during centrifugation and are floating on the surface, try not to capture them when collecting the supernatant.

2. Load remaining supernatant to the column. Centrifuge for 1 min, 14000 rcf. Discard the flow-through.

3) Column wash

1. Add 10 ml WB1 to the column. Centrifuge for 1 min, 14000 rcf. Discard the flow-through.
2. Add 10 ml WB2 to the column. Centrifuge for 1 min, 14000 rcf. Discard the flow-through.

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

3. Centrifuge column for 3 min, 14000 rcf to completely remove the WB2.

4) DNA elution

1. Transfer the column into a new collecting tube (included in the kit).
2. Apply 1 ml EB directly to the center of the column membrane. Incubate for 3 min at room temperature (15-25 °C). Centrifuge for 1 min, 14000 rcf.

3. Transfer eluate to a new clear 1.5 ml microtube (not included).
Note: 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).
4. 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.

Protocol 3. Precipitation of plasmid DNA using high-speed microcentrifuge (speed not less than 12000 rcf)

For precipitation of plasmid DNA from 800–1000 µl of eluate, use 2 ml microtubes. Measure the concentration of plasmid DNA by UV spectrometry or fluorimetry methods before starting the precipitation procedures.

1. Add 0.1 volumes PS1 to the eluted sample.
2. Mix by pipetting or vortex for 3 – 5 s.

Example: if sample volume after elution is 1000 µl then add 100 µl PS1. A little excess of PS1 does not affect final yields.

3. Add 0.7 volumes PS2. Mix by pipetting or vortex for 3 – 5 s.

Example: if sample volume is 1000 µl, PS1 volume is 100 µl, then add 770 µl PS2. A little excess of PS2 does not affect final yields.

4. Centrifuge for 15 min, 12000 rcf at +4 °C. Carefully discard the supernatant, do not touch the pellet.

Important! The pellet may be transparent or slightly white. Remember or mark the position of microtube in centrifuge.

5. Add 500 µl WS to the pellet. Carefully invert the tube 1-2 times to wash the pellet.

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

6. Centrifuge for 10 min, 12000 rcf at +4 °C. Carefully discard the supernatant, do not touch the pellet.

Important! The pellet may be transparent or slightly white. Remember or mark the position of microtube in centrifuge.

7. Air-dry the pellet for 10-15 min at room temperature (15-25 °C).

Important! Do not overdry the pellet as it makes DNA difficult to redissolve.

8. Dissolve the pellet of plasmid DNA in EB. It is recommended to add 0.8-1 µl EB to 1 µg DNA used for precipitation. In this case the resulting concentration of plasmid DNA will be 1 - 1.25 µg/µl (1000 - 1250 ng/µl).

Important! Increase volume of EB if it is not enough to cover the whole pellet.

9. Store DNA solution 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.

Note: DNA yield after precipitation is 80-100%.

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).
- RNAse A (Cat. No. ER-500).

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.