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Column cells, tissues, blood genomic DNA isolation kit (DU)

Cat. No. DU-10, DU-50, DU-250

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 January 2024.

Description

The kit is designed for isolation and purification of genomic DNA from the following samples:

1. Animal cell cultures;
2. Gram-negative and gram-positive bacterial cell cultures;
3. Animal and plant tissues;
4. Blood.

The method of DNA isolation is based on the selective binding of nucleic acids from a lysed sample on a silica-gel membrane, followed by washing and elution of the purified DNA. Column capacity up to 50 μg of DNA per one column. DNA yield depends on a sample type.

The isolated DNA can be used for PCR, nick-translation, and other genetic engineering applications.

Kit contents

	DU-10 10 preps	DU-50 50 preps	DU-250 250 preps	
			Var. 1	Var. 2
Lysis buffer LB	8 ml	40 ml	4x50 ml	2x100 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
Collection tubes and spin columns	10 pcs	50 pcs	250 pcs	250 pcs

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

Safety Information

Caution! Lysis LB and wash WB1 buffers contain chaotropic salt solution, which is irritating and toxic. Buffers are toxic in contact with skin and insides, causing burns. You must follow the rules of general and personal safety when working with the kit.
In case of a contact with skin, wash immediately with plenty of water and detergents. Visit a doctor if necessary.
Warning! When working with biological fluids, wear disposable gloves, since material may potentially be infected and capable of storing or transmitting HIV, hepatitis virus or any other infection for a long time. All used materials should be disinfected and disposed in accordance with local requirements.

Operation

Components: LB, WB1, WB2, EB are stable after opening the bottles at temperatures from +15°C to +25°C throughout the entire shelf life, provided the bottles are sufficiently sealed.

Operation conditions

Ambient temperature from +15 to +25°C;
Relative air humidity no more than 80%;
Atmospheric pressure 630 – 800 mm. Hg.

Equipment and reagents to be supplied by user

- Microcentrifuge for 1.5-2 ml tubes, speed 10000 rcf;
- Vortex;
- Single-channel variable volume micropipettes with disposable tips;
- Disposable gloves;
- 1.5 ml microcentrifuge tubes;
- Ethanol, 96-100%;
- Optional:
  Lysozyme for DNA isolation from gram-positive bacteria;

Before starting the procedures

Preparing the WB2 buffer

- **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.

Prepare a 50 mg/ml lysozyme solution (optional)

When isolating DNA from gram-positive bacteria, prepare a lysozyme solution with a concentration of 50 mg/ml using a lysozyme dissolving buffer:

Option 1. 50 мM Tris-HCl (pH 8), 10 мM EDTA (pH 8), 50% glycerol.

Option 2. 50 мM Tris-HCl (pH 8), 10 мM EDTA (pH 8).

To dissolve lysozyme in buffer, vortex the mixture thoroughly and incubate at room temperature for 30 min on a shaker or stirring occasionally till completely dissolved.

Store the solution of lysozyme in buffer (option 1) at -20°C 6 months.

Do not store the solution of lysozyme in buffer (option 2).

Lysozyme and lysozyme dissolving buffer are not included.

DNA isolation protocol

1) Preparing and lysing the samples

Animal or bacterial cells, lymphocytes

1. Resuspend the cell pellet in 50 µl PBS.

Note: do not use more than $3*10^6$ animal cells or lymphocytes and not more than $1*10^8$ bacterial cells.

Note: when isolating DNA from gram-positive bacteria add 30 µl of lysozyme solution (50 mg/ml in lysozyme dissolving buffer). Incubate for 10 min at room temperature (15-25°C).

2. Add 600 µl LB.
3. Mix thoroughly by pipetting or vortexing, avoid foaming.
4. Discard droplets by short centrifugation.
5. Incubate for 10 min at room temperature (15-25°C).

Animal and plant tissues

1. Place a sample of tissue in a clean 1.5-2.0 ml microtube.

Note: do not use more than 20-30 mg of animal tissue (more than 10-15 mg of spleen), not more than 50-100 mg of fresh plant tissue, not more than 20-30 mg of dry plant tissue.

2. Add 600 µl LB. Homogenize the sample.
3. Discard droplets by short centrifugation.
4. Incubate for 10 min at room temperature (15-25°C).
5. Centrifuge the lysate for 1 min, 10000 rcf. Gently transfer the supernatant to the column (**see "Column loading"**) or into new 1.5 – 2 ml tube (not included).

Whole blood

1. Add 200 µl of whole blood into a clean 1.5-2.0 ml microtube.
2. Add 750 µl LB.

Note: If DNA is isolated from 100 µl of whole blood also 750 µl of LB should be used.

3. Mix thoroughly by pipetting or vortexing.
4. Discard droplets by short centrifugation.
5. Incubate for 10 min at room temperature (15-25°C).

2) Column loading

1. Transfer not more than 800 µl of the lysate or the supernatant (**see "Animal and plant tissues"**) to the column.
2. Centrifuge for 30 s, 10000 rcf. Discard the flow-through.

Note: if there is residual solution in a column after centrifugation, repeat the centrifugation step by increasing the speed or time of centrifugation before loading next portion of the sample.

Note: If the sample volume is more than 800 µl, transfer the excess on the same column and repeat centrifugation.

3) Column wash

1. Add 500 µl WB1 to the column. Centrifuge for 30 s, 10000 rcf. Discard the flow-through.
2. Add 500 µl WB2 to the column. Centrifuge for 30 s, 10000 rcf. Discard the flow-through.

Note: ensure that ethanol was added to the WB2.

3. Centrifuge column for 3 min, 10000 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 analisys

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} ~ 1.8 – 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

All components of the kit can be stored at room temperature (15-25 °C). See expiration date on the package label.

Shipping

All components of the kit are shipped at room temperature (15-25 °C).