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Column animal and bacterial cells DNA isolation kit (D-Cells)

Cat. No. D-Cells-10, D-Cells-50, D-Cells-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 February 2024.

Description

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

1. Animal cell cultures;
2. Gram-negative and gram-positive bacterial cell cultures.

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. Sample lysis occurs in the presence of proteinase K.
The isolated DNA can be used for PCR, nick-translation, and other genetic engineering applications.

Contents

	D-Cells-10 10 preps	D-Cells-50 50 preps	D-Cells-250 250 preps	
			Var. 1	Var. 2
PBS	2 ml	8.5 ml	45 ml	45 ml
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	5.5 ml	30 ml	3x50 ml	2x75 ml
Elution buffer EB	5 ml	15 ml	60 ml	60 ml
Proteinase K	240 µl	1.2 ml	5x1.2 ml	5x1.2 ml
Lysozyme dissolving buffer	400 µl	2 ml	10 ml	10 ml
Collection tubes and spin columns	10 pcs	50 pcs	250 pcs	250 pcs

The D-Cells-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 if it comes in contact with skin or inside, causing burns. When working, always wear a suitable lab coat, disposable gloves, and protective goggles.
Caution! Wash buffers WB1 and WB2 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.
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: PBS, LB, WB1, WB2, EB, lysozyme dissolving buffer and proteinase K 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.
Caution! Do not store the mixture of lysis buffer LB and proteinase K.

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

- A dry block heater maintaining temperature 56 °C °C;
- 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;
- Optional:
  Lysozyme for DNA isolation from gram-positive bacteria.

DNA isolation protocol

1) Preparing and lysing the samples

Animal cell cultures. Monolayer cultures

1. Remove cells from the surface of the culture plastic by the method used in the laboratory or the standard method recommended for this culture.
2. Transfer the cell suspension sample (up to 2-3*10^6 cells) into a microtube.
3. Precipitate cells by centrifugation for 3 min, 1000 rcf. Carefully remove the supernatant. Resuspend the cell pellet in 150 µl of PBS.
4. Add 150 µl LB and 20 µl proteinase K.
5. Vortex for 5-10 s. Discard droplets by short centrifugation.
6. Incubate for 10 min at 56 °C.
7. Add 500 µl LB.
8. Vortex for 5-10 s. Discard droplets by short centrifugation.
9. Incubate for 5 min at room temperature.
10. Continue with section (2) "Column loading".

Animal cell cultures. Monolayer cultures. Cultural plates

When working with 12-, 24-, 96-well plates or culture plastic with a similar cell area, lysis is allowed directly in the well.

1. Remove the culture medium from the well of the plate.
2. For a 12-well plate, add 400 µl LB to the well;

For 24- or 96-well plate, add 200 µl LB to the well.

3. Incubate for 3-5 min. Gently mix the suspension by pipetting, avoiding foaming. Make sure that the cells have detached.
4. Transfer the sample to a microtube. Add 20 µl proteinase K.
5. Vortex for 5-10 s. Discard droplets by short centrifugation.
6. Incubate for 10 min at 56 °C.
7. Add 400 µl LB for 12-well plate or 600 µl LB for 24- or 96-well plate samples.
8. Vortex for 5-10 s. Discard droplets by short centrifugation. Incubate for 5 min at room temperature.
9. Continue with section (2) "Column loading".

Animal cell cultures. Suspended cell cultures.

1. Transfer the cell suspension sample (up to 2-3*10^6 cells) into a microtube.
2. Precipitate cells by centrifugation for 3 min, 1000 rcf. Carefully remove the supernatant. Resuspend the cell pellet in 150 µl of PBS.
3. Add 150 µl LB and 20 µl proteinase K.
4. Vortex for 5-10 s. Discard droplets by short centrifugation.
5. Incubate for 10 min at 56 °C.
6. Add 500 µl LB.

7. Vortex for 5-10 s. Discard droplets by short centrifugation. Incubate for 5 min at room temperature.
8. Continue with section (2) "Column loading".

Bacterial cell cultures. Gram-negative bacteria.

1. Transfer 0.5-2 ml of overnight cell culture suspension (up to $1*10^8$ cells) into a microtube.

Note: when working with *E.Coli* cell cultures, it is recommended to use up to 500 µl of overnight cell culture.

2. Precipitate cells by centrifugation for 1 min, 10000 rcf. Carefully remove the supernatant. Resuspend the cell pellet in 150 µl of PBS.
3. Add 150 µl LB and 20 µl proteinase K.
4. Vortex for 5-10 s. Discard droplets by short centrifugation.
5. Incubate for 10 min at 56 °C.
6. Add 500 µl LB.
7. Vortex for 5-10 s. Discard droplets by short centrifugation. Incubate for 5 min at room temperature.
8. Continue with section (2) "Column loading".

Bacterial cell cultures. Gram-positive bacteria.

Preparation of a lysozyme solution:

- Add the required volume of lysozyme dissolving buffer (included; 50 mM Tris-HCl (pH 8), 10 mM EDTA (pH 8), 50% glycerol) to the lysozyme powder to obtain solution with 50 mg/ml concentration.
- Mix thoroughly, using a vortex.
- Incubate for 30 min at room temperature (15-25°C), mixing periodically until completely dissolved.
- Store at -20 °C.

1. Transfer 0.5-2 ml of overnight cell culture suspension (up to $1*10^8$ cells) into a microtube.
2. Precipitate cells by centrifugation for 1 min, 10000 rcf. Carefully remove the supernatant. Resuspend the cell pellet in 150 µl of PBS.
3. Add 30 µl of lysozyme solution (50 mg/ml), dissolved in a lysozyme dissolving buffer.

Note: It is recommended to use a fresh solution of lysozyme and store it up to 6 months at -20 °C. Lysozyme is not included in the kit.

4. Vortex for 5-10 s. Discard droplets by short centrifugation. Incubate for 10 min at room temperature.
5. Add 50 µl LB and 20 µl proteinase K.
6. Vortex for 5 – 10 s. Discard droplets by short centrifugation.
7. Incubate for 10 min at 56 °C.
8. Add 600 µl LB.

9. Vortex for 5-10 s. Discard droplets by short centrifugation. Incubate for 5 min at room temperature.
10. Continue with section (2) "Column loading".

2) Column loading

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

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

Note: If there is residual solution in a column after centrifugation, repeat the centrifugation step by increasing the speed and time of centrifugation before "Column wash".

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.
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.

Note: recommended elution volume is 100 µl.

- 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} ~ 1.8 – 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

The kit can be stored at room temperature (15-25 °C). Proteinase K 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.