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D-Cells Kit for DNA Isolation from Animal and Bacterial Cells

Catalog number: D-Cells-10, D- Cells-50, D- Cells250

Attention!

We regularly improve the protocol for the reagent handling, so please use the protocol provided with the product.
These kits are intended to be used for scientific research purposes only.
The protocol was updated on July 15, 2022.

Product Description

The kit is intended for express isolation of DNA from the following samples:

1. animal cell culture
2. gram-negative and gram-positive bacterial cell cultures

An operation principle of the kit is based on the selective sorption of nucleic acids from a previously lysed sample on a silicon membrane, followed by washing and elution of the purified product. Sample lysis occurs in the presence of proteinase K.

The isolated DNA can be used for PCR, nick-translation, sequencing, etc.

Kit Components

	D-Cells-10 10 extractions	D-Cells-50 50 extractions	D-Cells-250 250 extractions
PBS	1.1 ml	5.5 ml	30 ml
Buffer solution for lysis (LB)	8 ml	40 ml	2x100 ml
Buffer solution for washing (WB1)	5.5 ml	30 ml	2x70 ml
Buffer solution for washing (WB2)	5.5 ml	30 ml	2x70 ml
Buffer solution for elution (EB)	5 ml	15 ml	60 ml
Proteinase K solution	240 µl	1.2 ml	5x1.2 ml
TE buffer	400 µl	2 ml	10 ml
Test-tubes for filtrate collection with columns for the sample sorption	10 pcs	50 pcs	250 pcs

Safety Precautions

Caution! Buffer solutions for lysis LB and for washing WB1 contain irritant and toxic chaotropic salt solution. While working, it is necessary to follow the rules of general and personal safety precautions. The solutions are toxic in contact with skin and if swallowed and causes chemical burns.

Caution! Buffer solutions for washing WB1 and WB2 contain isopropyl alcohol, which is irritating and toxic. Do not work with the solution in the close proximity to open flame. In case of skin contact: wash immediately with plenty of water and soap (detergent). Get medical attention if necessary.

Operation

The components of PBS, LB, WB1, WB2, EB 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.

Attention! Do not heat the kit above 25°C. The violation of the storage and transportation temperature regime reduces the activity of proteinase K and the isolation efficiency.

Attention! Do not store the mixture of lysis buffer LB and proteinase K.

Operating Conditions

Ambient temperature: 15 – 25 °C;

Relative air humidity: no more than 80 %;

Atmospheric pressure: 630 – 800 mm Hg.

Required Equipment and Materials (not included into the kit)

- A dry block heater maintaining temperature of 56°C ±1°C;
- A centrifuge for microcentrifuge tubes (1.5-2 ml), rotation speed 12000 rcf;
- A vibration mixer (Vortex);
- Single-channel variable volume micropipettes with disposable tips;
- Lysozyme solution (50 mg/ml) in a TE buffer (0.01 M Tris-HCl, 0.001 M EDTA, pH 8.0);
- Rubber gloves;
- Microcentrifuge tubes (1.5 ml).

DNA Isolation Protocol

1) Sample Preparation and Lysis

– 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 cell culture.
2. Transfer the cell suspension sample (no more than 2-3*10^6 cells) into a disposable microtube.
3. Pellet cells by centrifugation for 3 min at 1000 rcf. Carefully remove the supernatant. Resuspend the cell pellet in 100 µl of PBS.
4. Add 100 µl of lysis buffer LB and 20 µl of proteinase K solution with a clean disposable tip.

5. Vortex the sample for 5-10 seconds. Dispose drops by short centrifugation.
6. Incubate for 10 min at 56 ºC.
7. Add 600 µl of lysis buffer LB to the sample.
8. Vortex the sample for 5-10 seconds. Dispose drops by short centrifugation. Incubate for 5 min at room temperature (15-25 ^{0}C).

- Animal cell cultures. Monolayer cultures. Cultural plates

When working with 12-, 24-, or 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 of lysis buffer LB to the well; for a 24- or 96-well plate, add 200 µl of lysis buffer LB to the well.
3. Incubate for 3-5 min. Mix carefully the contents of the well by pipetting, avoiding foaming. Make sure that the cells have detached from the cell.
4. Transfer the sample to a disposable microtube, using a clean disposable tip. Add 20 µl of proteinase K solution.
5. Vortex the sample for 5-10 seconds. Dispose drops by short centrifugation.
6. Incubate for 10 min at 56 ºC.
7. For a 12-well plate, add 400 µl of lysis buffer LB to the well; for a 24- or 96-well plate, add 600 µl of lysis buffer LB to the well.
8. Vortex the sample for 5-10 seconds. Dispose drops by short centrifugation. Incubate for 5 min at room temperature (15-25 ^{0}C).

- Animal Cell Cultures. Suspended Cell Cultures

1. Transfer the cell suspension sample (no more than 2-3*10^{6} cells) into a disposable microtube.
2. Pellet cells by centrifugation for 3 min at 1000 rcf. Carefully remove the supernatant. Resuspend the cell pellet in 100 µl of PBS.
3. Add 100 µl of lysis buffer LB and 20 µl of proteinase K solution with a clean disposable tip.
4. Vortex the sample for 5-10 seconds. Dispose drops by short centrifugation.
5. Incubate for 10 min at 56 ºC.
6. Add 600 µl of lysis buffer LB to the sample.
7. Vortex the sample for 5-10 seconds. Dispose drops by short centrifugation. Incubate for 5 min at room temperature (15-25 ^{0}C).

- Bacterial Cell Cultures. Gram-negative Bacteria

1. Transfer 0.5-2 ml of overnight cell culture suspension (no more than 1*10^{8} cells) into a disposable microtube.

Note: When working with E.Coli cell cultures, it is recommended to use no more than 500 µl of overnight cell culture.

2. Pellet cells by centrifugation for 1 min at 10000 rcf. Carefully remove the supernatant. Resuspend the cell pellet in 100 µl of PBS.
3. Add 100 µl of lysis buffer LB and 20 µl of proteinase K solution with a clean disposable tip.
4. Vortex the sample for 5-10 seconds. Dispose drops by short centrifugation.
5. Incubate for 10 min at 56 ºC.
6. Add 600 µl of lysis buffer LB to the sample.
7. Vortex the sample for 5-10 seconds. Dispose drops by short centrifugation. Incubate for 5 min at room temperature (15-25 ^{0}C).

- **Bacterial Cell Cultures. Gram-positive Bacteria**

Preparation of lysozyme solution:

- To dissolve lysozyme, add the required volume of buffer to the weighed quantity of lysozyme with a clean disposable tip.
- Mix thoroughly, using a vortex.
- Incubate for 30 min at room temperature (15-25 ^{0}C), mixing periodically until complete dissolution of lysozyme.
- Store at -20 ^{0}C.

1. Transfer 0.5-2 ml of overnight cell culture suspension (no more than 1*10^8 cells) into a disposable microtube.
2. Pellet cells by centrifugation for 1 min at 10000 rcf. Carefully remove the supernatant. Resuspend the cell pellet in 100 µl of PBS.
3. Add 30 µl of lysozyme solution (50 mg/ml) in a TE buffer (0.05 M Tris-HCl (pH 8), 0.01 M EDTA (pH 8.0), 50 % glycerine) using a clean disposable tip.

Note: Lysozyme is not included into the kit. Lysozyme dissolution buffer is supplied with the kit. It is recommended to use a fresh solution of lysozyme and store it for no more than 2 weeks at -20 ^{0}C.

4. Vortex the sample for 5-10 seconds. Dispose drops by short centrifugation. Incubate for 10 min at room temperature (15-25 ^{0}C).
5. Add 100 µl of lysis buffer LB and 20 µl of proteinase K solution with a clean disposable tip.
6. Vortex the sample for 5-10 seconds. Dispose drops by short centrifugation.
7. Incubate for 10 min at 56 ºC.
8. Add 600 µl of lysis buffer LB to the sample.
9. Vortex the sample for 5-10 seconds. Dispose drops by short centrifugation. Incubate for 5 min at room temperature (15-25 ^{0}C).

2) Application to the Column

1. Transfer 800 µl of lysate to the column. Close the column cap tightly.
2. Centrifuge for 30 sec at 12000 rcf. Remove the filtrate.

Note: If the lysate volume is greater than 800 µl, reapply the excess solution to the same column and repeat centrifugation. If a portion of the solution remains on the column after centrifugation, repeat centrifugation with increased time and/or speed of centrifugation without adding a new portion of LB buffer.

3) Washing the Column

1. Apply 500 µl of washing buffer WB1 to the column. Centrifuge for 30 sec at 12000 rcf. Remove the filtrate.
2. Apply 500 µl of washing buffer WB2 to the column. Centrifuge for 30 sec at 12000 rcf. Remove the filtrate.
3. Centrifuge the column for 3 min at 12000 rcf until the complete removal of the WB2 buffer.

4) DNA Elution

1. Transfer the column to a clean 1.5 ml microcentrifuge tube. Press the column firmly against the tube.
2. Apply 60-200 µl of elution buffer EB to the center of the column filter. Incubate for 3 min at room temperature (15-25 °C).
3. Centrifuge for 1 min at 12000 rcf.

Note: With a smaller volume of elution buffer, the total DNA yield may decrease, but the DNA concentration in the resulting solution will be higher.

The elution buffer EB contains 0.01 M Tris • HCl (pH 8.0).
The sample can also be eluted with a TE buffer (0.01 M Tris-HCl, 0.001 M EDTA, pH 8.0-8.5) or weak-alkaline water (pH 8.0-8.5), treated with DEPC.
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.
Attention! The presence of EDTA in the eluate may adversely affect further enzymatic reactions.

Analysis of Isolated DNA

The integrity of the isolated DNA can be checked by gel electrophoresis in 1% agar gel.

The amount of isolated DNA can be estimated, using UV spectrometry.
Typical absorption maximum for DNA occurs at a wavelength λ = 260 nm.
One can calculate the concentration of DNA (μg/ml), using the following formula:
A_{260} * dilution * 50 μg/ml.
The typical ratio of optical densities at 260 nm and 280 nm is A_{260} / A_{280} ~ 1.7-2.0.

Storage Requirements

DNA isolation kit can be stored at room temperature (15-25 °C) for 12 months.

Storage Requirements

The kit, including proteinase K, should be transported at temperatures from 15 to 25 °C. Transportation is allowed at a temperature not exceeding 25 °C for 14 days.

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