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Kit for Express DNA Isolation

Cat. No. D-Tissues-10, D-Tissues-50, D-Tissues-250

Important!

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 August 25, 2022.

Description

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

1. Human and animal cell lines;
2. Bacteria cell lines;
3. Samples of buccal epithelium. Saliva.

An operation principle of the kit is based on rapid cell lysis using proteinase K. The isolated DNA can be used for PCR.

Kit Components

	FL-bio-100 from 100 extractions*	FL-bio-200 from 200 extractions*
Buffer solution for lysis (LB)	62 ml	2x62 ml
Proteinase K	450 µl	2x450 µl

Safety Precautions

Caution! Buffer solution for lysis (LB) contains a mixture of detergents and may cause irritation in case of contact with the eyes. The buffer is non-toxic. In case of eye contact: wash immediately with plenty of water and soap (detergent). Get medical attention if necessary.

Operation

Fast LB components 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. The mixture of lysis buffer LB and proteinase K can be stored for 14 days at a temperature not exceeding 2-8°C.

Operation conditions

Ambient temperature: 15 – 25 °C;
Relative air humidity: No more than 80 %;
Atmospheric pressure: 630 – 800 mm Hg

Required Equipment and Materials (materials not included in the kit)

- A dry block heater maintaining temperature of 95°C ±1°C;
- Single-channel variable volume micropipettes;
- A vibration mixer (a vortex);
- A centrifuge for microcentrifuge tubes (1.5-2 ml), with a rotation speed of 3000 rcf;
- Rubber gloves;
- Microcentrifuge tubes;
- Disposable pipette tips;
- Sodium phosphate buffer or normal saline (0.9% NaCl).
- Lysozyme solution (50 mg/ml) in a TE buffer (0.01 M Tris-HCl, 0.001 M EDTA, pH 8.0)

DNA Isolation Protocols. Sample Preparation and Lysis

Human and Animal Cell Lines

1) Determine the number of cells in the wells of the plate.
2) Remove the culture medium from the plates with cell lines. Add the required amount of lysis buffer LB, using a single-channel micropipette (see table 1). Mix by pipetting and leave for 1 minute.
3) Wait until the cells are completely detached from the plate and transfer to a microcentrifuge tube. Use a clean disposable tip to mix the content of the wells.
4) Add the required amount of proteinase K to the tube (see Table 1), using a clean disposable tip. Vortex the sample for 10 seconds.
5) Dispose drops by short centrifugation.
6) Incubate for 10 min at 56 ºC.
7) After that, incubate for 10 min at 95 ºC.
8) If there are some insoluble particles, centrifuge samples at 3000 rcf for 1 minute. The sample is suitable for analysis by PCR.

Table 1. Reagents Ratio for Lysis of Human and Animal Cell Lines

Number of cells per a well	Volume of buffer solution LB	Proteinase K solution, 20 mg/ml
Up to 100,000 pcs	100 µl	0.5 µl
100,000 – 250.000 pcs	150 µl	1 µl
250.000 – 500.000 pcs	200 µl	1.5 µl
500.000 – 1.000.000 pcs	400 µl	2 µl
More than 1.000.000 pcs	600 µl	4 µl

Bacteria Cell Line

1) Determine the number of cells. In case of using a cell suspension, pellet the cells by centrifugation and remove the supernatant.
   Note. To increase the DNA yield from gram-positive bacteria, prepare a solution of lysozyme (not included in the kit) with the concentration of 50 mg/ml in a TE buffer (0.01 M Tris-HCl, 0.001 M EDTA, pH 8.0). Add 200 µl of TE buffer to the tube containing pellet. Resuspend the cells and add 30 µl of lysozyme solution (50 mg/ml). Incubate for 10 min at 15-25 ºC.
2) In a separate test tube (a vial), mix the required amount of buffer solution LB and proteinase K (see table 2).

3) Add the required amount of lysis buffer LB using a single-channel micropipette (see table 1). Leave it for 1 minute. Add the required amount of proteinase K to the tube with a clean disposable tip (see table 1). Vortex the sample for 10 seconds.
4) Dispose drops by short centrifugation.
5) Incubate for 10 min at 56 ºC.
6) After that, incubate for 10 min at 95 ºC.
7) If there are some insoluble particles, centrifuge samples at 3000 rcf for 1 minute. The sample is suitable for analysis by PCR.

Table 2. Reagents Ratio for Lysis of Bacteria Cell Lines

Number of cells per a well	Volume of buffer solution LB	Proteinase K solution, 20 mg/ml
Up to 100,000 pcs	100 µl	0.5 µl
100,000 – 250.000 pcs	150 µl	1 µl
250.000 – 500.000 pcs	200 µl	1.5 µl
500.000 – 1.000.000 pcs	400 µl	2 µl
More than 1.000.000 pcs	600 µl	4 µl

Samples of buccal Epithelium. Saliva

1) Add 400 µl of buffer solution LB to a microtube with a clean disposable tip and add 2 µl of proteinase K solution. Vortex the sample for 10 seconds. Dispose drops by short centrifugation.
2) Introduce samples of buccal epithelium (when introducing a sample from a swab, it is necessary to squeeze the swab well against the tube wall. In case of using saliva, take at least 200 µl of saliva).
3) Vortex the content of the microtubes for 10 seconds.
4) Dispose drops by short centrifugation.
5) Incubate for 10 min at 56 ºC.
6) After that, incubate for 10 min at 95 ºC.
7) If there are some insoluble particles, centrifuge samples at 3000 rcf for 1 minute. The sample is suitable for analysis by PCR.

Analysis of Isolated DNA

The samples obtained are suitable for analysis by PCR.

Note: Fast LB solution contains a mixture of detergents, which does not allow using these samples for subsequent analysis via gel electrophoresis or UV spectrometry. For further analysis, we recommend using a kit for nucleic acid precipitation.

Storage

Buffer solution (LB) can be stored at room temperature (15-25 °C) for 12 months.

Proteinase K solution should be stored at –18 - –24 °C (in freezer) for 12 months.

Shipping

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

Attention! Do not heat the kit above 25°C. the violation of the storage and transportation temperature requirements reduces the effectiveness of lysis.

Note: Do not store the mixture of lysis buffer LB and proteinase K for more than 14 days.