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Kit for DNA Isolation from Animal Tissues

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 July 22,2022.

Description

The kit is intended for isolation and purification of DNA from animal tissues.

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-Tissues-10 10 extractions	D-Tissues -50 50 extractions	D-Tissues -250 250 extractions
PBS	2.5 ml	12 ml	60 ml
Buffer solution for lysis (LB)	0.6 ml	3 ml	15 ml
Buffer for application to the column (BB)	8.5 ml	45 ml	2x110 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
Test-tubes for filtrate collection with columns for the sample sorption	10 pcs	50 pcs	250 pcs

Safety information

Caution! Buffer solutions for application to the column BB 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 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.

Caution! Disposable rubber gloves should be worn while working with biological samples, because the test material is potentially infectious and capable to retain or transmit HIV, hepatitis virus or any other viral pathogen for a long time. Disinfect all used materials in accordance with the requirements of Disinfection and Sterilization Guideline МУ-287-113.

Operation

The components of PBS, LB, BB, 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 expose the kit to direct sunlight and 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.

Operation conditions

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

Equipment and reagents to be supplied by user

- A dry block heater maintaining temperature up to 56°C ±1°C;
- A centrifuge for microcentrifuge tubes (1.5-2 ml), with a rotation speed of 12000 rcf;
- A vibration mixer (Vortex);
- Single-channel variable volume micropipettes with disposable tips;
- Rubber gloves;
- Microcentrifuge tubes (1.5 ml).

DNA Isolation Protocol

Attention! It is a good practice to carry out isolation from fresh tissue samples. If tissue samples need to be stored **for long periods**, it is recommended to use **an RNA stabilizer (St-100)** or analogous reagents.

Sample Preparation and Lysis

1) Sample Types

For DNA isolation, it is acceptable to use samples in RNA stabilizer (St-100), either fresh, chilled samples, or samples frozen in liquid nitrogen. It is recommended to choose the method of samples homogenization before starting to work (see Paragraph 9.1.2).

1. Samples in RNA Stabilizer
   Remove the sample from the stabilizer and weigh It. Use no more than 30 mg of tissue (no more than 10 mg of spleen) for DNA isolation.
2. Fresh Samples
   Freshly obtained samples should be weighed immediately. Avoid exposing the sample to air or storing it without a stabilizer. Use no more than 30 mg of tissue (no more than 10 mg of spleen) for DNA isolation.
3. Frozen Samples
   Frozen samples must be weighed. The use of frozen samples stored for more than 1 year at −20°C is not recommended. Use no more than 30 mg of tissue (no more than 10 mg of spleen) for DNA isolation.
4. Sample Storage Guideline

Samples in RNA stabilizer (St-100)

- Storage for 3 days at 37°C
- Storage for 1 week at temperature from 15 to 25°C
- Storage for 1 month at temperature from 2 to 8°C
- Storage for 1 year at temperature from −24 to −18°C
- Storage no more than 5 years at temperature from −80 to −70°

Fresh samples

- Immediate use
- Storage no more than 1 year at temperature from −24 to −18°C
- Storage no more than 5 years at temperature from −80 to −70°

Frozen samples

- Storage no more than 1 year at temperature from –24 to –18°C
- Storage no more than 5 years at temperature from –80 to –70°

2) Samples Homogenization

1. Homogenization Using Liquid Nitrogen
   Place the tissue sample in liquid nitrogen. Thoroughly grind the sample in a mortar and pestle. Transfer the ground sample in liquid nitrogen to a disposable 1.5 ml microtube. Wait until the nitrogen has evaporated, but do not allow the tissue to melt. Add 200 µl of PBS.
2. Homogenization Using a Mechanical Homogenizer
   Add 200 µl of PBS to the tissue sample. Close the microtube tightly. Grind using a mechanical tissue homogenizer such as FastPrep-24™ 5G (MP Biomedicals), TissueRuptor II (QIAGEN).
   Note: When using a homogenization matrix (powder or beads), PBS should completely cover the homogenization matrix and the tissue sample. If the amount of PBS is insufficient, then use an additional aliquot of PBS or saline (additional aliquots of these solutions are not included in the kit).
3. Homogenization Using a Lancet
   Cut the tissue sample into small fragments. Place the sample in a 1.5 ml disposable microtube, add 200 µl of PBS.
   Note: The smaller the tissue fragments, the faster the lysis of the sample will take place.

3) Sample Lysis

1. Add 20 µl of proteinase K solution and 50 µl of lysis buffer LB with a clean disposable tip to ground tissue sample in 200 µl of PBS.
2. Vortex the sample for 5-10 seconds. Dispose drops by short centrifugation.
3. Incubate for 0.5-3 h at 56 ºC until tissue dissolves. Mix repeatedly by a hand or using a vortex.

Note: The lysis time depends on the type of tissue and the degree of sample grinding. The lysis procedure of the sample may be left overnight. For more efficient lysis, it is recommended to mix the sample periodically (2-3 times per hour) or use a thermostatically controlled shaker.

4. After lysis is complete, add 700 µl of buffer BB (buffer for application to the column) to the sample. Mix vigorously by a hand or using a vortex. Incubate for 5 min at room temperature.
5. Centrifuge the sample for 10 min at 12000 rcf.

Application to the Column

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

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.

DNA Elution

1) Transfer the column to a clean 1.5-2 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).
   Centrifuge for 1 min at 12000 rcf.

Note: It is recommended to use 100 µl of elution buffer EB. With a decrease in the buffer volume, a decrease in the total DNA yield is possible. The minimum volume of elution buffer EB is 60 µl.

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.

Note: The quantity and quality of the isolated DNA depend on the type of biomaterial, conditions and duration of storage, as well as the lysis duration. The approximate amount of isolated DNA for different types of tissues is listed in Table 2.

Table 2. The amount of DNA isolated from 10 mg of tissue (using M. Musculus as an example).

Tissue type	Amount of DNA
Liver	From 5 to 20 µg
Heart	From 1 to 10 µg
Lungs	From 10 to 30 µg
Kidneys	From 5 to 20 µg
Spleen	From 20 to 80 µg

Storage

DNA isolation kit can be stored at room temperature (15-25 °C) for 12 months. Proteinase K solution should be stored at –18 - –24 °C for 12 months.

Shipping

The kit (including proteinase K) should be transported at room temperature (from 15 to 25 °C). Transportation is allowed at room temperature for 14 days.