



Limited liability company

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D-Swabs Kit for DNA Isolation from Swab Samples and Scrapings of Epithelial Cells and Saliva.

Cat. No. D-Swabs-10, D-Swabs-50, D-Swabs-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 19,2022.

Description

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

1. Buccal epithelium;
2. Swab samples from mucous membranes;
3. Saliva;
4. Transport media samples with swab samples from mucous membranes;
5. Swab samples from the surface.

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.

The isolated DNA can be used for PCR and nick-translation.

Contents

	D-swabs-10 10 extractions	D-swabs -50 50 extractions	D-swabs -250 250 extractions
Transport media ES	7 ml	2x15 ml	2x70 ml
Buffer solution for lysis (LB)	7 ml	2x15 ml	2x70 ml
Buffer solution for washing (WB1)	5.5 ml	2x15 ml	2x70 ml
Buffer solution for washing (WB2)	5.5 ml	2x15 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 τ	250 pcs

Safety information

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 ES, 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.

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), rotation speed 10000 rcf;
- A vibration mixer (a vortex);
- Single channel variable volume micropipettes with disposable tips;
- Rubber gloves;
- Microcentrifuge tubes (1.5 ml).

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 MY-287-113.

Sample Collection and Preparation

1) Preparing and lysing the samples Buccal Epithelium

1. Before sampling the buccal epithelium, one should refrain from eating at least 2 hours before the procedure. The collection of buccal epithelium should be carried out with a clean disposable sterile probe (a swab).
2. Rinse the mouth 3 times with clean, warm water before sampling. Do not use toothpaste, mouthwash, chlorhexidine or alcohol solutions.
3. To collect a sample of epithelial cells from the inner surface of the cheek, make 10 brushing actions with a swab, rubbing the cheek with a light pressure. And then immediately place the swab into a clean disposable 1.5 ml plastic tube or into a tube with 500 µl of ES transport medium.

Attention! Do not touch a cotton swab with hand! Do not touch other objects with a swab!

To store a sample of buccal epithelium, cut a swab so that the end with the collected epithelium falls into the tube. The other end of the swab, which was held by the hand, should be disposed.

Note: The material can be stored in a freezer at $-20\text{ }^{\circ}\text{C}$ not more than for 1 month without the addition of ES (in a dry form).

Before the analysis, incubate the tube with a cotton swab for 5-10 minutes at room temperature. If the swab was stored dry, add 500 µl of ES transport medium beforehand. Vortex the sample after incubation. Then follow the basic protocol.

2) Swab Samples from Mucous Membranes

Attention! Trained and qualified medical personnel should carry out swab sampling from such mucous membranes as urogenital, vaginal, cervical canal, nasopharynx, pharynx, ear, rectum, discharge from the eye, etc. Selected samples should be stored in a specialized transport medium.

It is not allowed to mix transport media for different samples with each other. When working with these samples, follow the basic protocol.

3) Saliva

Before sampling the saliva, one should refrain from eating, drinking or smoking for 30 minutes before the procedure.

Rinse the mouth with water and wait for 10 minutes before sampling.

To collect saliva, use clean 1.5 ml tubes. Collect 200 µl of saliva into a test tube. Saliva samples can be stored at temperature $4-8^{\circ}\text{C}$ no longer than one day. The material can

be stored in a freezer at -20°C no longer than 1 month. Frozen material must be thawed at room temperature before use.

When working with these samples, follow the basic protocol

4) Transport Media Samples with Swab Samples from Mucous Membranes

Samples placed in transport media produced by other manufacturers do not require additional preparation. Samples storage should be carried out depending on the requirements for the sample type and the type of transport medium (see the description for the transport medium). When working with these samples, follow the basic protocol

5) Swab Samples from Surfaces

To take swab samples from the surface, use a clean probe (a swab) moistened in a transport medium or saline solution. Swipe over the examined surface with a moistened swab, drawing a grid 5×5 cm in size. The swab must be rotated during the sampling. After the collection, transfer the swab into a tube with $500\ \mu\text{l}$ of ES transport medium for 5-10 minutes. Carefully wash the swab with a solution and press it. Dispose of the used swab.

When working with these samples, follow the basic protocol

DNA Isolation Protocol

1) Sample Preparation and Lysis

1. Buccal Epithelium

- Add $500\ \mu\text{l}$ of lysis buffer LB with a clean disposable tip to the tube containing the sample in $200\ \mu\text{l}$ of transport media.
- Add $20\ \mu\text{l}$ of proteinase K solution, using a clean disposable tip.
- Vortex the sample for 5-10 seconds.
- Dispose drops by short centrifugation.
- Incubate for 10 min at 56°C .

2. Swab Samples from Mucous Membranes

- Collect up to $200\ \mu\text{l}$ of the sample in transport medium to a disposable tube. Add $400\ \mu\text{l}$ of lysis buffer LB with a clean disposable tip.
- Add $20\ \mu\text{l}$ of proteinase K solution, using a clean disposable tip.
- Vortex the sample for 5-10 seconds.
- Dispose drops by short centrifugation.
- Incubate for 10 min at 56°C .

3. Saliva

- Collect up to $200\ \mu\text{l}$ of the sample to a disposable tube. Add $400\ \mu\text{l}$ of lysis buffer LB with a clean disposable tip.
- Add $20\ \mu\text{l}$ of proteinase K solution, using a clean disposable tip.
- Vortex the sample for 5-10 seconds.
- Dispose drops by short centrifugation.
- Incubate for 10 min at 56°C .

4. Transport Media Samples with Swab Samples from Mucous Membranes

- Collect up to 200 µl of the sample to a disposable tube. Add 400 µl of lysis buffer LB with a clean disposable tip.
- Add 20 µl of proteinase K solution, using a clean disposable tip.
- Vortex the sample for 5-10 seconds.
- Dispose drops by short centrifugation.
- Incubate for 10 min at 56 °C.

5. Swab Samples from Surfaces

- Add 400 µl of lysis buffer LB with a clean disposable tip to the tube containing the sample in 200 µl of transport media
- Add 20 µl of proteinase K solution, using a clean disposable tip.
- Vortex the sample for 5-10 seconds.
- Dispose drops by short centrifugation.
- Incubate for 10 min at 56 °C.

2) Application to the Column

1. Transfer lysate to the column. Close the column cap tightly.
2. Centrifuge for 30 sec at 10000 rcf. Remove the filtrate.

3) Washing the Column

1. Apply 500 µl of washing buffer WB1 to the column. Centrifuge for 30 sec at 10000 rcf. Remove the filtrate.
2. Apply 500 µl of washing buffer WB2 to the column. Centrifuge for 30 sec at 10000 rcf. Remove the filtrate.
3. Centrifuge the column for 3 min at 10000 rcf until the complete removal of the WB2 buffer.

4) DNA Elution

1. Transfer the column to a clean 1.5-2 ml microcentrifuge tube. Press the column firmly against the tube.
2. Apply 100 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 10000 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

Isolated DNA can be analyzed by real-time PCR or classical PCR.

Note! The amount of DNA isolated from swab samples is usually below the limit of quantification by agarose gel electrophoresis.

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