Limited liability company
«Biolabmix»
TIN 5408278957 CAT 540801001
630090, Novosibirsk obl., Novosibirsk,
st. Injenernaya, building № 28
Tel/Fax: +7(383)363-51-91, Tel: +7(383)363-22-40
E-mail: sales@biolabmix.ru

Column swabs DNA isolation kit (D-Swabs)

Cat. No. D-Swabs-10, D-Swabs-50, D-Swabs-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. Buccal epithelium;
2. Swab samples from mucous membranes;
3. Saliva;
4. Swab samples from mucous membranes in transport medium;
5. Swab samples from the surface.

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-Swabs-10 10 preps	D-Swabs-50 50 preps	D-Swabs-250 250 preps	
			Var. 1	Var. 2
Transport medium ES	7 ml	40 ml	3x50 ml	2x75 ml
Lysis buffer LB	7 ml	40 ml	3x50 ml	2x75 ml
Wash buffer WB1	5.5 ml	40 ml	3x50 ml	2x75 ml
Wash buffer WB2	5.5 ml	40 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
Collection tubes and spin columns	10 pcs	50 pcs	250 pcs	250 pcs

The D-Swabs-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: ES, LB, WB1, WB2, EB 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.

Sample collection and preparation

1) Buccal epithelium

1. Before sampling the buccal epithelium, one should refrain from eating for 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 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.
4. Immediately place the swab into a clean 1.5 ml microtube or into a tube with 500 µl of transport medium ES.

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

Note: to store a sample of buccal epithelium, cut a swab so that the end with the collected buccal epithelium falls into the tube.

Note: the sample can be stored in a freezer at -20 °C not more than 1 month without the addition of ES (in a dry form).

5. Before the analysis, incubate the tube with a cotton swab for 5-10 min at room temperature (15-25 °C). If the swab was stored dry, add 500 µl of transport medium ES preliminary. Vortex the sample after incubation.
6. Follow the "**DNA isolation protocol**", starting from the section **«Preparing and lysing the samples»** → **«Buccal epithelium»**.

2) Swab samples from mucous membranes

Attention! Sampling from mucous membranes such as urogenital, vaginal, cervical canal, nasopharynx, pharynx, ear, rectum, discharge from eye, etc. should be carried out by trained and qualified medical personnel. Taken samples should be stored in a specialized transport medium.

Do not mix transport medium from different samples with each other.

Follow the "**DNA isolation protocol**", starting from the section **«Preparing and lysing the samples»** → **«Swab samples from mucous membranes»**.

3) Saliva

1. Before sampling the saliva, one should refrain from eating, drinking and smoking for 30 minutes before the procedure.
2. Rinse mouth with water and wait for 10 minutes before sampling. To collect saliva, use clear 1.5 ml microtubes.
3. Collect 200 µl of saliva into a test tube. Saliva samples can be stored at 2-8 °C not longer than one day or at -20 °C for 1 month.
4. If using frozen material, thaw it at room temperature.
5. Follow the "**DNA isolation protocol**", starting from the section **«Preparing and lysing the samples»** → **«Saliva»**.

4) Swab samples from mucous membranes in transport medium

1. Samples placed in transport media produced by other manufacturers do not require additional preparation.
2. Sample 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).
3. Follow the "**DNA isolation protocol**", starting from the section **«Preparing and lysing the samples»** → **« Swab samples from mucous membranes in transport medium »**.

5) Swab samples from surfaces

1. To take swab samples from the surface, use a clean probe (a swab) moistened in a transport medium or saline solution.
2. Swipe over the examined surface, drawing a 5x5 cm grid. Rotate the swab during the sampling.
3. Place the swab into a clean 1.5 ml microtube or into a tube with 500 μl of transport medium ES. Incubate the tube with a cotton swab for 5-10 min at room temperature (15-25 °C).
4. Carefully wash the swab with a solution and squeeze it into a tube. Dispose the used swab.
5. Follow the "**DNA isolation protocol**", starting from the section **«Preparing and lysing the samples»** → **«Swab samples from surfaces»**.

DNA isolation protocol

1) Preparing and lysing the samples

Buccal epithelium

1. Add 500 µl LB to the tube with the sample in 200 µl of transport medium.
2. Add 20 µl proteinase K.
3. Vortex for 5-10 s.
4. Discard droplets by short centrifugation.
5. Incubate for 10 min at 56 °C.
6. Continue with section (2) "Column loading".

Swab samples from mucous membranes

1. Collect up to 200 µl of the sample in transport medium to a 1.5 ml microtube. Add 400 µl LB.
2. Add 20 µl proteinase K.
3. Vortex for 5-10 s.
4. Discard droplets by short centrifugation.
5. Incubate for 10 min at 56 °C.
6. Continue with section (2) "Column loading".

Saliva

1. Collect up to 200 µl of the sample to a 1.5 ml microtube. Add 400 µl LB.
2. Add 20 µl proteinase K.
3. Vortex for 5-10 s.
4. Discard droplets by short centrifugation.
5. Incubate for 10 min at 56 °C.
6. Continue with section (2) "Column loading".

Swab samples from mucous membranes in transport medium

1. Collect up to 200 µl of the sample to a 1.5 ml microtube. Add 400 µl LB.
2. Add 20 µl proteinase K.
3. Vortex for 5-10 s.
4. Discard droplets by short centrifugation.
5. Incubate for 10 min at 56 °C.
6. Continue with section (2) "Column loading".

Swab samples from surfaces

1. Collect up to 200 µl of the sample in transport medium to a 1.5 ml microtube. Add 400 µl LB.
2. Add 20 µl proteinase K.
3. Vortex for 5-10 s.
4. Discard droplets by short centrifugation.
5. Incubate for 10 min at 56 °C.

6. Continue with section (2) “Column loading”.

2) Column loading

1. Transfer lysate to the column.
2. Centrifuge for 30 s, 10000 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, 10000 rcf. Discard the flow-through.
2. Add 500 µl WB2 to the column. Centrifuge for 30 s, 10000 rcf. Discard the flow-through.
3. Centrifuge column for 3 min, 10000 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

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 detection by gel electrophoresis or UV-spectrometry.

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.