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Column blood DNA isolation kit (D-Blood)

Cat. No. D-Blood-10, D-Blood-50, D-Blood-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. Whole blood collected into disposable blood collection tubes with the following anticoagulant agents: K_3EDTA, sodium citrate, CPDA, heparin;
2. Blood plasma;
3. Blood serum;
4. Cryoprecipitate;
5. Leukocyte suspension;
6. Cerebrospinal Fluid or liquor.

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-Blood-10 10 preps	D-Blood-50 50 preps	D-Blood-250 250 preps	
			Var. 1	Var. 2
Lysis buffer LB	5 ml	25 ml	2x60 ml	120 ml
Wash buffer WB1	5.5 ml	30 ml	3x50 ml	2x75 ml
Wash buffer WB2	5.5 ml	30 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-Blood-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: 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;
- Microcentrifuge for 1.5-2 ml tubes, speed 10000 rcf;
- Vortex;
- Single-channel variable volume micropipettes with disposable tips;
- Sodium chloride solution (9 g/l NaCl) or saline;
- Disposable gloves;
- 1.5 ml microcentrifuge tubes.

DNA isolation protocol

1) Preparing and lysing the samples

Whole blood

1. Gently mix the tube with whole blood, avoid the sample to separate into plasma and cell fraction. Transfer 200 µl of whole blood to a microtube.
2. Add 400 µl LB.
3. Add 20 µl proteinase K.
4. Vortex for 5-10 s.
5. Discard droplets by short centrifugation.
6. Incubate for 10 min at 56 °C.

Blood plasma, blood serum, leukocytes

- **Leukocyte-rich plasma**

Centrifuge a whole blood sample placed into a tube with anticoagulants (K_3EDTA or sodium citrate) for 3 min, 1500 rcf. Collect plasma.

- **Leukocyte-poor plasma**

Centrifuge a whole blood sample placed into a tube with anticoagulants (K_3EDTA or sodium citrate) for 3 min, 3000 rcf. Collect plasma.

- **Blood serum**

Use heparinized blood. Leave the tube with blood at room temperature for 15 min. Then, use a thin glass rod (or a pipette tip) to gently separate the clot from the walls of the tube without damaging the cells. Centrifuge for 10 min at 3000 rcf. Separate the serum from the clot immediately after centrifugation.

Note: it is not recommended to centrifuge plasma and serum samples above 3000 rcf as this may lead to hemolysis of the samples.

- **Leukocyte-rich suspension**

Transfer 1000 µl of whole blood into a microtube, centrifuge for 5 min, 3000 rcf at room temperature. After centrifugation, 3 different fractions can be distinguished: the upper transparent layer is plasma; the intermediate layer is a buffy coat suspension containing concentrated leukocytes; the bottom layer is concentrated erythrocytes.

Gently collect the intermediate layer, without capturing the upper and lower layers, and transfer into a clean microtube. Wash the sample with 500 µl of saline. Centrifuge for 5 min, 3000 rcf at room temperature. Collect the intermediate layer and transfer it to another microtube. Suspend cells in 200 µl of saline.

1. Transfer 200 µl of the sample to a microtube (blood plasma, blood serum or leukocyte-rich suspension).
2. Add 400 µl LB.
3. Add 20 µl proteinase K.
4. Vortex for 5-10 s.

5. Discard droplets by short centrifugation.
6. Incubate for 10 min at 56 °C.

Liquor

1. Transfer 500 µl of whole blood into a microtube.
2. Centrifuge for 10 min, 5000 rcf at room temperature.
3. Remove the supernatant leaving the pellet and approximately 50 µl of liquid in the tube.
4. Add 500 µl of saline to the pellet. Vortex for 5-10 s.
5. Centrifuge for 10 min, 3000 rcf at room temperature.
6. Remove the supernatant leaving the pellet and approximately 200 µl of liquid in the tube.
7. Add 400 µl LB.
8. Add 20 µl proteinase K.
9. Vortex for 5-10 s.
10. Discard droplets by short centrifugation.
11. Incubate for 10 min at 56 °C.

Dry blood stain

Use specialized filter paper.

1. Press out 3 circles (with a diameter not less than 1.2 mm) from the dry blood stain. Place the cut-out circles in 1.5 ml tubes.
2. Add 400 µl LB.
3. Add 20 µl proteinase K.
4. Vortex for 5-10 s.
5. Discard droplets by short centrifugation.
6. Incubate for 10 min at 56 °C.

2) Column loading

1. Transfer the 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

DNA can be analyzed by gel electrophoresis in 1% agarose gel.
The amount of isolated DNA can be estimated using UV spectrometry.
The maximum of absorption for DNA corresponds to λ = 260 nm.
DNA concentration (μg/ml) can be calculated using the following formula:
A_{260} * dilution * 50 μg/ml.
Typical optical density ratios are A_{260}/A_{280} ~ 1.8 – 2.0.
Note: DNA isolated from blood plasma and blood serum is usually below the limit of detection by agarose gel electrophoresis or UV-spectrometry.

Additional ordering Information

- Buffers for agarose gel electrophoresis: TAE (Cat. No. BE-DNA-500, BE-DNA-1000), TBE (Cat. No. TBE-500).
- Ethidium bromide (Cat. No. EtBr-10) for nucleic acid visualisation gel electrophoresis.
- Nucleic acids loading buffers (Cat. No. D-3001, D-3002, D-3003) for agarose gel electrophoresis.
- DNA ladders
  (Cat. No. S-8000, S-8100, S-8103, S-8055, S-8250, S-8150).

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.