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Magnetic blood DNA isolation kit

Cat. No. MagBlood-100

Manual and automatic methods for DNA isolation

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 whole blood collected into disposable blood collection tubes with the following anticoagulant agents: K3EDTA, sodium citrate, CPDA, heparin.

The method of DNA isolation is based on the selective binding of nucleic acids from a lysed sample on magnetic particles formed with iron and silica oxides, followed by washing and elution of the purified DNA.

It is possible to extract DNA both manually by using a magnetic stand and automatically by using the automatic nucleic acid purification systems **Auto-Pure96 (Allsheng).**

The isolated DNA can be used for PCR.

Contents

Cat. No.	MagBlood-100 100 preps
Lysis buffer LB	50 ml
Binding buffer BB	2x40 ml
Wash buffer WB	2x60 ml
Elution buffer EB	15 ml
Magnetic particles M	4x1500 µl

Safety Information

Caution! Binding buffer BB and wash buffer WB 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, BB, WB, EB and M 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 binding buffer BB and magnetic particles M.

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

Manual protocol

- Dry block incubator capable to reach temperature 60 °C;
- Magnetic rack for 1.5-2 ml microtubes;
- Vortex;
- Single-channel variable volume micropipettes with disposable tips;
- Disposable gloves;
- 1.5 ml microcentrifuge tubes.

Automatic protocol

- 96 deepwell plate, V-shaped bottom, 2 ml wells, 5 pcs;
- 96 tip comb for deep-well magnets, 1 pcs.

DNA isolation protocol. Manual protocol

1.1) Preparing and lysing the samples. Without proteinase K

1. Gently mix the tube with whole blood, avoid the sample to separate into plasma and cell fraction.
2. Transfer 200 µl of whole blood to a microtube.
3. Add 400 µl LB.
4. Mix thoroughly by pipetting or vortexing.
5. Discard droplets by short centrifugation.
6. Incubate for 10 min at 60 °C.

1.2) Preparing and lysing the samples. With proteinase K

1. Gently mix the tube with whole blood, avoid the sample to separate into plasma and cell fraction.
2. Take 200 µl of whole blood to a clear microtube.
3. Add 200 µl LB.
4. Add 20 µl proteinase K (20 mg/ml).

Note: Proteinase K solution is not included in the Magnetic blood DNA isolation kit.

5. Mix thoroughly by pipetting or vortexing.
5. Discard droplets by short centrifugation.
6. Incubate for 10 min at 60 °C.

2) DNA binding to magnetic particles

1. Mix magnetic particles suspension thoroughly by stirring manually or vortexing to obtain homogeneous suspension.
2. Add an equal volume of BB to the lysate. Mix thoroughly by pipetting by or vortexing to obtain homogeneous mixture.

Example 1. If 200 µl of the whole blood and 400 µl of lysis buffer were used, than required volume of BB is 600 µl.

Example 2. If 200 µl of the whole blood, 200 µl of lysis buffer, 20 µl of proteinase K were used, than required volume of BB is 420 µl.

3. Add 50 µl of magnetic particles suspension to the sample and immediately mix by pipetting or vortexing to obtain homogeneous suspension.
4. Incubate for 10 min at room temperature (15-25°C).
5. Place the tube with sample to magnetic rack. Incubate for 5 min.

Note: Make sure that the magnetic particles have collected on the tube wall. If a significant fraction of particles remains in the solution, increase the incubation time.

6. Discard the supernatant while the tube in magnetic rack. Don't disturb magnetic particles at the tube wall.

3) Magnetic particles washing

1. Add 500 µl of WB to the tube. Mix thoroughly by pipetting or vortexing to obtain homogeneous suspension.

2. Place the tube in magnetic rack. Incubate for 5 min.

Note: Make sure that the magnetic particles have collected on the tube wall. If a significant fraction of particles remains in the solution, increase the incubation time.

3. Discard the supernatant while the tube in magnetic rack. Don't disturb magnetic particles at the tube wall.
4. Repeat steps 1-3.
5. Dry the tube with magnetic particles in air at 15-25 °C for 5-15 minutes or until completely dry.

4) DNA elution

1. Add 50-100 µl of EB. Mix thoroughly by pipetting or by vortex to obtain homogeneous suspension. Incubate for 5 minutes at 15-25 °C.

Note: 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).

2. Place the tube in magnetic rack. Incubate for 5 min.

Note: Make sure that the magnetic particles have collected on the tube wall. If a significant fraction of particles remains in the solution, increase the incubation time.

3. Transfer the supernatant into a new tube. Don't disturb magnetic particles at the tube wall.
4. 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.

Note: DNA can be analyzed by gel electrophoresis in 1% agarose gel.

Note: The concentration of isolated DNA cannot be determined using UV spectrometry; fluorimetric methods for determining DNA concentration are recommended.

DNA isolation protocol. Automatic protocol. Auto-Pure96 (Allsheng)

1) Preparing and lysing the samples

1. The plate at position #1. Comb. Place a comb in a clean 96-well plate.
2. The plate at position #2. Lysis. Add the components listed below to the well of the plate. Choose one of the variants.

Variant 1. 200 µl LB, then 200 µl whole blood.

Variant 2. 300 µl LB, then 100 µl whole blood.

Variant 3. 200 µl LB, 20 µl of proteinase K solution (20 mg/ml), then 200 µl of whole blood.

Note: proteinase K solution is not included in the Magnetic blood DNA isolation kit.

Note: proteinase K can increase DNA yield by 2-4 times. It is possible to prepare a mixture of LB and proteinase K. Do not store this mixture.

3. The plate at position #3. Washing. Add 500 µl of the washing buffer WB to the well of the plate.
4. The plate at position #4. Washing. Add 500 µl of the washing buffer WB to the well of the plate.
5. The plate at position #8. DNA elution. Add 100 µl of the elution buffer EB to the well of the plate.

Note: elution buffer EB is 0.01 M Tris•HCl (pH 8.0).

6. Run the program «MB_lys» on the station Auto-Prep96 (Allsheng).

Note: During the program «MB_lys» samples are lysed.

7. At the end of the program "MB_lys", remove the plate from position #2, containing lysed samples.

2) Binding and purification of the samples on magnetic particles

1. In a clean tube, prepare a mixture of binding buffer BB and magnetic particles. Mix by pipetting or vortexing to obtain homogeneous mixture. Prepare this mixture after finishing the program "MB_lys" program; do not store the mixture.

1 prep. 450 µl binding buffer BB, 50 µl magnetic particles M.

100 preps (+10%). 50 ml binding buffer BB, 5.5 ml magnetic particles M, total volume 55.5 ml.

Note: It is recommended to increase the desired volume by 10% when working with multiply samples.

2. Add 500 µl of a mixture of BB and M to the wells of the plate contained lysed samples at position #2.
3. Return the plate with lysate, BB and M mixture to position #2.
4. Run the program "MB_pur" on the Auto-Pure 96 (Allsheng) station.

Note: During the program «MB_pur» DNA purification is occurred.

5. At the end of the program «MB_pur», the isolated DNA will be in the plate at position #8.
4. 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.

Note: DNA can be analyzed by gel electrophoresis in 1% agarose gel.
Note: The concentration of isolated DNA cannot be determined using UV spectrometry; fluorimetric methods for determining DNA concentration are recommended.

Note: The files with the programs «MB_lys» and «MB_pur» for the Auto-Prep96 (Allsheng) station can be obtained in the following ways:

- download it yourself on the website of Biolabmix LLC (www.biolabmix.ru). Enter the product catalog number (MagBlood-100) in the search bar;
- contact the sales department of Biolabmix LLC (sales@biolabmix.ru).

Storage

The kit can be stored at room temperature (15-25 °C). Magnetic particles M should be stored at 2 °C to 8 °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.