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Kit for DNA isolation from plant material on magnetic particles

Cat. no. MagPlants-100, MagPlants-1200

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 09/17/2024.

Description

The kit is designed for the isolation and purification of DNA from pre-homogenized plant samples.
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

	MagPlants-100 100 preps	MagPlants-1200 1200 preps
Homogenization buffer GB	25 ml	12x25 ml
Lysis buffer LB	2x45 ml	24x45 ml
Sedimentation buffer SB	12 ml	12x45 ml
Binding buffer BB	22 ml	12x22 ml
Wash buffer WB1	2x45 ml	24x45 ml
Wash buffer WB2	2x45 ml	24x45 ml
Wash buffer WB3	2x10 ml	24x10 ml
Elution buffer EB	15 ml	12x15 ml
Magnetic particles M	2x1.1 ml	24x1.1 ml

Safety information

Caution! Buffers GB, LB, SB, BB, WB1, WB2 contain solutions of irritating and toxic substances. General and personal safety regulations must be observed during work. Toxic in contact with skin and inhalation, irritating.

Caution. Buffer WB1 contains isopropanol, which is irritating and toxic. Do not work with the solution in the vicinity of an open flame.

In case of skin contact, wash immediately with plenty of water and detergent (detergent). Seek medical attention if necessary.

Attention! When working with the kit, wear disposable rubber gloves and follow safety precautions when working with substances that are irritating to the skin and mucous membranes.

Operation

Components: GB, LB, SB, BB, WB1, WB2, WB3, EB, M are stable throughout the shelf life provided storage conditions (see on the package) and sufficient sealing of the vials are observed.

Attention! Do not heat the set above the temperature of +25°C, non-compliance with the temperature conditions of storage and transportation reduces the effectiveness of the release.

Operation conditions

Ambient temperature from +15 to +25 °C;
Relative air humidity less than 80 %;
Atmosphere pressure 630 – 800 mmHg.

Materials and equipment necessary for work

Manual method

- Magnetic rack for 1.5-2 ml microtubes
- Dry block incubator capable to reach temperature 60 °C
- Vortex
- Microtube centrifuge for 1.5-2 ml, speed 10000 rcf
- Microcentrifuge tubes for 1.5-2 ml
- Single-channel variable volume micropipettes with disposable tips
- Disposable rubber gloves

Automatic method

- Deepwell plate with V-shaped bottom, V-wells for 2 ml, 6 pcs.
- Magnetic rod tips or comb, 1 pc.
- Single-channel/multichannel variable volume pipettes and their tips
- Disposable rubber gloves

Before starting the procedures

Preparation of the WB3 buffer.

- 1 isolation, 700 µl. To 140 µl of WB3 buffer (concentrate) add 560 µl of ethanol (96-100%).
- 1 vial. To 10 ml of WB3 buffer (concentrate) add 40 ml of ethanol (96-100%) to obtain 50 ml of WB3 buffer.

Close the lid tightly after adding ethanol. It is recommended to add ethanol to the aliquots of the WB4, since ethanol may partially evaporate when storing the buffer for several months.

DNA extraction protocol. Manual method

1) Sample preparation

- The recommended sample weight depends on the species and age of the plant, but should not exceed 200 mg per isolate.
- For homogenization at automated stations or with disposable pestles, add 200 µl of GB homogenization buffer to the sample suspension.
- Homogenization in liquid nitrogen can be performed without the use of GB homogenization buffer.
- After homogenization, make sure that no large unhomogenized particles remain in the sample.

Important: Ensure that you use disposable homogenization systems or completely remove the sample from the homogenization system after use to avoid contamination of subsequent samples.

- When working with dried samples, it is necessary to consider the method of preparation of the material. In the case of working with collection and old samples, the quantity and quality of DNA extracted may be reduced due to temperature or time degradation. If this is the case, make sure that you have the opportunity to isolate from different parts of the plant and perform the isolation in several repetitions.
- In the presence of contaminants (soil, insects, etc.), samples should be rinsed in purified water, then the remaining liquid should be removed with disposable paper towels.

Important: The composition of the plant is very diverse and depends strongly on the species and the place where it grows. In addition to a dense cell wall composed of polysaccharides, plants contain large amounts of polyphenolic compounds and acidic components that often lead to a decrease in the quality and quantity of nucleic acids released. The protocol using LB lysis buffer allows proteins and polysaccharides to be removed by precipitation with PS buffer.

- If it is necessary to place tissue samples in long-term storage, it is recommended to use RNA stabilizer (St-100) or similar reagents. RNA stabilizer has been tested with leaves, needles and young roots.

2) Lysis of samples

1. Add 800 µl LB to the microtube with the ground sample.
2. Stir the sample on the vortex for 5-10 s. Discharge the droplets by short centrifugation.

Optional. RNA impurity removal

Attention! When working with samples with high RNA concentrations or further work sensitive to the presence of RNA impurity, it is recommended to remove RNA using

RNase A (Cat. No. ER-500). To remove RNA impurity, add 5 µl of RNase A to the lysate. Stir the sample on a vortex for 5-10 s. Discard the droplets by short centrifugation.

3. Incubate for 10 minutes at 60 °C.
4. Stir the sample on the vortex for 5-10 s.

Caution. When working with automatic homogenizers that use a homogenization matrix, remove the matrix from the sample before adding PS buffer. For this purpose, centrifuge the samples for 2 min, 12000 rcf and then collect the entire liquid volume and transfer it to a new tube.

5. Add 100 µl of SB precipitation buffer. Stir the sample on the vortex for 5-10 s.
6. Cool on ice. (Incubate for 10 minutes at +4°C).
7. Stir the sample on the vortex for 5-10 s.
8. Centrifuge for 5 min, 12000 rcf. Transfer 600 µl of supernatant to a clean tube.

3) DNA binding to magnetic particles

1. Resuspend the magnetic particles M by stirring manually or on a vortex until a homogeneous suspension is formed.
2. Add 200 µl of BB sorption buffer to the lysate. Mix thoroughly by pipetting or on a vortex until a homogeneous mixture is obtained.
3. Add 20 µl of magnetic particle suspension, mix immediately by pipetting or on a vortex until a homogeneous suspension is obtained.

4 Incubate for 5 min at room temperature. Mix thoroughly by pipetting or on a vortex until a homogeneous suspension is obtained.

5. Place the sample tube in the magnetic rack. Incubate for 2 minutes.

Note: make sure that the magnetic particles are collected on the wall of the test tube. If a significant fraction of particles remain in the solution, increase the incubation time.

6. Without removing the tube from the magnetic rack, carefully withdraw the supernatant without touching the magnetic particles.

4) Magnetic particles washing

1. Add 800 µl of WB1 Wash Buffer to the tube containing the magnetic particles. Mix thoroughly by pipetting or on a vortex until a homogeneous suspension is obtained.
2. Place the sample tube in the magnetic rack. Incubate for 2 minutes.

Note: make sure that the magnetic particles are collected on the wall of the test tube. If a significant fraction of particles remain in the solution, increase the incubation time.

3. Without removing the tube from the magnetic rack, carefully withdraw the supernatant without touching the magnetic particles.

4. Add 800 µl of WB2 wash buffer to the tube with magnetic particles. Mix thoroughly by pipetting or on a vortex until a homogeneous suspension is obtained.
5. Place the sample tube in the magnetic rack. Incubate for 2 minutes.
Note: make sure that the magnetic particles are collected on the wall of the test tube. If a significant fraction of particles remain in the solution, increase the incubation time.
6. Without removing the tube from the magnetic rack, carefully withdraw the supernatant without touching the magnetic particles.
7. Add 800 µl of WB3 wash buffer to the tube with magnetic particles. Mix thoroughly by pipetting or on a vortex until a homogeneous suspension is obtained.
Note: Remember to add ethanol to the WB3 buffer beforehand.
8. Place the sample tube in the magnetic rack. Incubate for 2 minutes.
Note: make sure that the magnetic particles are collected on the wall of the test tube. If a significant fraction of particles remain in the solution, increase the incubation time.
10. Without removing the tube from the magnetic rack, carefully withdraw the supernatant without touching the magnetic particles.
11. Without removing the tube from the magnetic rack dry the tube with magnetic particles in the air for 5-15 minutes at 15-25 °C or until completely dry (alcohol odor is eliminated).

5) DNA elution
Elute DNA using Option 1 (using a magnetic rack) or Option 2 (using a centrifuge). Elution of DNA using Option 2 removes extraneous particles from the eluate and produces a purer DNA solution, which is not always possible using a magnetic rack for manual DNA extraction.

5.1) DNA elution. Option 1: Using a magnetic rack
1. Add 100 µl of EB elution buffer to the tube containing the magnetic particles. Mix thoroughly by pipetting or on a vortex until a homogeneous suspension is obtained. Incubate for 3 minutes at 60 °C.
Note: The elution buffer 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 water (pH 8.0-8.5, pH adjusted with NaOH solution).
2. Place the sample tube in the 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.
Important: If foreign particles are present in the eluate, centrifuge the DNA sample for 3 min, 12000 rcf. Transfer the supernatant containing DNA to a clean tube.

4. Store the eluate containing DNA at -20°C. For long-term storage it is recommended to add EDTA (pH 8) to a final concentration of 0.1-1 mM.

Note: EDTA can inhibit enzymatic reactions such as PCR.

Note: The isolated DNA can be analyzed by gel electrophoresis in 1% agarose gel, by UV spectrometry, or by PCR.

5.2) DNA elution. Option 2. Using centrifugation

1. Add 100 µl of EB elution buffer to the tube containing the magnetic particles. Mix thoroughly by pipetting or on a vortex until a homogeneous suspension is obtained. Incubate for 3 minutes at 60 °C.

Note: The elution buffer 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 water (pH 8.0-8.5, pH adjusted with NaOH).

2. Centrifuge for 3 min, 12000 rcf. Transfer the supernatant containing DNA to a clean tube.
3. Store the eluate containing DNA at -20°C. For long-term storage it is recommended to add EDTA (pH 8) to a final concentration of 0.1-1 mM.

Note: EDTA can inhibit enzymatic reactions such as PCR.

Note: The maximum possible DNA yield is 5 µg and depends on the amount and type of sample. The extracted DNA can be analyzed by gel electrophoresis in 1% agarose gel, by UV spectrometry or by PCR.

DNA and RNA isolation protocol. Automatic method. Auto-Pure 96 (Allsheng)

1) Sample Lysis.

Attention! Homogenization and lysis of the samples takes place separately, not at the extraction station.

Note: General guidelines for sample preparation can be found in the previous section "DNA extraction protocol. Manual method".

- The recommended sample weight depends on the species and age of the plant, but should not exceed 200 mg per isolate.
- For homogenization at automated stations or with disposable pestles, add 200 µl of GB homogenization buffer to the sample suspension.
- Homogenization in liquid nitrogen can be performed without the use of GB homogenization buffer.
- After homogenization, make sure that no large unhomogenized particles remain in the sample.

1. Add 800 µl LB to the microtube with the ground sample.
2. Stir the sample on the vortex for 5-10 s. Discard the droplets by short centrifugation.

Optional. RNA impurity removal

Attention! When working with samples with high RNA concentrations or further work sensitive to the presence of RNA impurity, it is recommended to remove RNA using RNase A (Cat. No. ER-500). To remove RNA impurity, add 5 µl of RNase A to the lysate. Stir the sample on a vortex for 5-10 s. Discard the droplets by short centrifugation.

3. Incubate for 10 minutes at 60 °C.
4. Stir the sample on the vortex for 5-10 s.

Caution. When working with automatic homogenizers that use a homogenization matrix, remove the matrix from the sample before adding SB buffer. For this purpose, centrifuge the samples for 2 min, 12000 rcf and then collect the entire liquid volume and transfer to a new tube.

5. Add 100 µl of PS precipitation buffer. Stir the sample on the vortex for 5-10 s.
6. Cool on ice. (Incubate for 10 minutes at +4°C).
7. Stir the sample on the vortex for 5-10 s.
8. Centrifuge for 5 min, 12000 rcf. Transfer 500 µl of the supernatant to the plate for the automatic extraction station.

2) Station preparation.

1. The plate at position #1. Comb. Place a comb in a clean 96-well plate.
2. The plate at position #2. Lysis. Add 150 µl of BB sorption buffer to the well of the plate.

3. The plate at position #3. Wash #1. Add 700 µl of WB1 Wash Buffer to the well of the plate.
4. The plate at position #4. Wash #2. Add 700 µl of WB2 wash buffer to the well of the plate.
5. The plate at position #5. Wash #3. Add 800 µl of WB3 wash buffer to the well of the plate.

Note: Remember to add ethanol to the WB3 buffer beforehand.

6. The plate at position #8. DNA elution. Add 100 µl of EB elution buffer to the well of the plate.

Note: Elution buffer - 0.01 M Tris-HCl (pH 8.0).

3) Binding and purification of the samples on magnetic particles

1. Add 500 µl of lysed sample, 150 µl of BB sorption buffer, 20 µl of M. magnetic particles to the well of plate #2.

Note: It is allowed to prepare a mixture of buffer for sorption of BB and magnetic particles beforehand. Mix on a vortex or by hand until a homogeneous suspension is obtained. Do not store the mixture of M magnetic particles and BB sorption buffer.

1 sample. 150 µl BB sorption buffer, 20 µl magnetic particles M.
100 samples (+10%). 22 ml of BB sorption buffer, 2.2 ml of magnetic particles M, total volume 24.2 ml.

2. Add 500 µl of lysate to the wells of the plate with the mixture of BB and magnetic particles at position 2.
3. Place the plate with lysate on position #2.
4. Start the program " MagPlant " at the Auto-Pure 96 station (Allsheng).

Note: nucleic acids are purified at this step.

5. After completion of the " MagPlant " program, the isolated DNA will be in the plate at position #8.
6. Store the eluate containing DNA at -20°C.

Note: The maximum possible DNA yield is 5 µg and depends on the amount and type of sample. The extracted DNA can be analyzed by gel electrophoresis in 1% agarose gel, by UV spectrometry or by PCR.

Important! You can obtain the program files " MagPlant.txt" for the Auto-Pure 96 (Allsheng) station by the following methods:

- download on the website of Biolabmix LLC https://biolabmix.ru/search/?q=MagPlants-100
- contact Biolabmix LLC sales department sales@biolabmix.ru

Additional reagents:

- Sterile pestles for homogenizing tissue samples in microtubes (Cat. No. pest-10).
- Buffers for agarose gel electrophoresis: Tris-acetate buffer (Cat. No. BE-DNA-500, BE-DNA-1000), Tris-borate buffer (Cat. No. TBE-500).
- Ethidium bromide solution (Cat. No. EtBr-10) for visualization of NK.
- Buffers for introducing DNA and RNA samples into the gel (Cat. Nos. D-3001, D-3002, D-3003).
- DNA molecular weight markers (Cat. No. S-8000, S-8100, S-8103, S-8055, S-8250, S-8150).
- RNase A (Cat. No. ER-500).
- BioMaster LR HS-PCR (2×) (Cat. No. MH040-100, MH040-400). For amplification of long DNA fragments from 0.2 to 30 bp.
- BioMaster LR HS-PCR-Color (2×) (Cat. No. MHC040-100, MHC040-400). For amplification of long DNA fragments from 0.2 to 30 bp.

Storage

Store magnetic particles at 2-8 °C. For expiration date, see the package.
All other components of the kit can be stored at room temperature (15-25 °C). See expiration date on the package label.

Shipping

All components of the kit are shipped at room temperature (15-25 °C). It is allowed to transport the set at a temperature not exceeding +25°C for 14 days.