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

D-Plants Kit for DNA Extraction from Plants

Catalog number: D-Plants-10, D-Plants-50, D-Plants-250

Attention!

We are regularly improving the protocol for the reagent handling, so please use the protocol provided with the product
The kits are intended for research purposes only.
The protocol was updated on June 9, 2023.

Product Description

The kit is designed to extract and purify DNA from the following samples:
1. Leaves, needles, stamens, green parts of plants
2. Roots, stems, bark
3. Fruits, berries, seeds
4. Mosses, lichens
5. Unicellular algae

An operation principle of the kit is based on selective sorption of nucleic acids from a pre-lyzed sample on a silicon dioxide membrane, subsequent washing and elution of the purified product.

The extracted DNA can be used for PCR, nick translation, sequencing, etc.

Kit Components

	D- Plants -10 10 extractions	D- Plants -50 50 extractions	D- Plants -250 250 extractions
Buffer solution for lysis (LB)	10 ml	50 ml	4x60 ml
Buffer solution for application to column (BB)	10 ml	50 ml	4x60 ml
Buffer solution for washing (WB)	12 ml	60 ml	5x50 ml
Buffer solution for elution (EB)	5 ml	15 ml	60 ml
RNase A solution	70	300	2x700
Filtrate collecting tubes and columns for sample sorption	10 pcs	50 pcs	250 pcs

Safety Precautions

Caution! The WB buffer contains isopropanol, having an irritating and toxic effect. Do not work with the solution in the close proximity to open flame.
In case of contact with the skin, rinse immediately with plenty of water and soap (detergent). Get medical attention if necessary.

Operation

Components: LB, WB, BB, EB are stable after opening the vials at a temperature from +15° C to +25° C during the entire shelf life, provided that the vials are sufficiently sealed. The RNase A solution is stable throughout the shelf life.
Attention! Do not heat the kit above a temperature of +25° C. Non-compliance with the temperature regime of storage and transportation reduces the activity of RNase A and the efficiency of extraction.

Operating Conditions

Ambient temperature is from +15 to +25° C;
The relative humidity of the air is below 80 %;
Atmospheric pressure is 630 – 800 mm Hg.

Required Equipment and Materials (not included into the kit)

- Dry block heater maintaining temperature of 65 ± 1° C;
- A centrifuge for micro-tubes for 1.5-2 ml capable of reaching a speed of at least 12000 rcf;
- Vibration mixer (Vortex);
- Single-channel variable volume pipettes and tips for them;
- Rubber gloves;
- MicroCentrifuge tubes (1.5 ml).

DNA Extraction Protocol

Preparation of samples

Attention! While working with dried samples, it is necessary to take into account how the material was prepared. In the case of working with collectible and old specimens, the quantity and quality of the DNA released can be reduced due to temperature or time degradation. In this case, make sure that you have the opportunity to extract from different parts of the plant and in several repetitions.
In case of contamination (soil, insects, etc.), the samples must be washed in purified water. Then remove the remaining moisture with disposable paper napkins.
Attention! While working with solid samples, make sure that you use a single-use homogenization system or completely remove the sample from the homogenization system after work to avoid contamination of subsequent samples.
Attention! If it is necessary to place tissue samples for **long-term storage**, it is recommended to use an **RNA stabilizer (St-100)** or similar reagents. The RNA stabilizer was tested with leaves, needles and young roots.

- Leaves, needles, stamens and similar parts of plants.
When working with soft parts of plants, it is recommended to use freshly cut samples, freeze-dried samples, or samples frozen in an RNA stabilizer (St-100).

Type of sample	Recommended sample weight for 1 extraction
Fresh samples	Not more than 100 mg
Frozen samples	Not more than 100 mg
Samples in the RNA stabilizer	Not more than 100 mg
Needles	Not more than 100 mg
Dry samples	No more than 30 mg
Stamens (in full)	No more than 50 mg

- Roots, stems, bark
When working with solid samples, it is recommended to use mechanical homogenizers or mills. DNA extraction is possible only from well-homogenized samples. A decreased quality of homogenization leads to a decrease in DNA yield.

Type of sample	Recommended sample weight for 1 extraction
Roots	No more than 30 mg
Bark	No more than 50 mg
Branches	No more than 50 mg

- Fruits, berries, seeds
When working with seed samples, it is recommended to use mechanical homogenizers or mills. DNA extraction is possible only from well-homogenized

samples. A decrease in the quality of homogenization leads to a decrease in DNA yield.
Soft samples can be dried with ethyl or isopropyl alcohol.

- Transfer weighed quantity of the sample to a 1.5 ml disposable test tube. Add 800 ml of alcohol to the weighed quantity of the sample. The sample should be thoroughly homogenized using a disposable pestle.
- Centrifuge for 1 min at 10000 rcf. Remove the supernatant without touching the precipitate.
- Re-add 800 ml of alcohol. Re-homogenize the sample, it is allowed to use the pestle repeatedly.
- Centrifuge for 1 min at 10000 rcf. Remove the supernatant without touching the precipitate.
- Dry the precipitate for 15 minutes at room temperature.

Type of sample	Recommended sample weight for 1 extraction
Fresh fruits	No more than 50 mg
Fresh berries	No more than 50 mg
Seeds	No more than 50 mg
Dry fruits	No more than 30 mg
Dry berries	No more than 30 mg

- Mosses, lichens

When working with mosses and lichens, make sure that the substrate to which the sample was attached is completely removed. Otherwise, the substrate DNA will be included in the extracted sample.

Type of sample	Recommended sample weight for 1 extraction
Moss	No more than 30 mg
Lichen	No more than 30 mg

Make sure that during lysis, the entire sample will be placed in the buffer for lysis (LB).

- Mushrooms

When working with dense samples of mushrooms, it is recommended to use mechanical homogenizers or mills. DNA extraction is possible only from well-homogenized samples. A decrease in the quality of homogenization leads to a decrease in DNA yield.
Soft samples can be dried with ethyl or isopropyl alcohol.

- Transfer the suspension to a 1.5 ml disposable test tube. Add 800 ml of alcohol to the suspension. The sample should be thoroughly homogenized using a disposable pestle.
- Centrifuge for 1 min at 10000 rcf. Remove the supernatant without touching the precipitate.
- Re-add 800 ml of alcohol. Re-homogenize the sample, it is allowed to use the pestle repeatedly.
- Centrifuge for 1 min at 10000 rcf. Remove the supernatant without touching the precipitate.
- Dry the precipitate for 15 minutes at room temperature.

Type of sample	Recommended sample weight for 1 extraction
Fresh mushrooms	No more than 30 mg
Mushrooms are dry	No more than 15 mg

- Unicellular algae

When working with algae suspensions, it is recommended to precipitate samples by centrifugation at 300-1000 rcf (depending on the type of a sample). If the sample is poorly precipitated during centrifugation, add 1/2 volume of 96% ethanol from the sample volume. The resulting precipitate should be washed with 80% ethanol. Centrifuge for 1 min at 10000 rcf. Remove the supernatant without touching the sediment.

Type of sample	Recommended sample weight for 1 extraction
Suspension of unicellular algae	No more than 200 µl

Lysis of Samples

- Homogenization in liquid nitrogen

- Take a sample of plant tissues. When working with samples in the RNA stabilizer (ST-100), drain the sample with a clean cloth. Weigh the sample.
- Completely cover the sample with liquid nitrogen, and thoroughly homogenize the sample.

Note: The quality of homogenization affects the DNA yield. It is recommended to obtain a homogeneous suspension of the sample.

- Quickly and carefully transfer the grinded sample to a clean test tube containing 750 ml of buffer for lysis (LB), and mix thoroughly.
- Incubate for 10 minutes at 65 °C. Cool the sample to room temperature.

- Homogenization using disposable pestles

- Take a sample of plant tissues. When working with samples in the RNA stabilizer (ST-100), drain the sample with a clean cloth. Weigh the sample.

- Quickly and carefully, transfer the sample to a clean test tube containing 750 ml of buffer for lysis (LB).
- Use a disposable pestle to grind thoroughly until a saturated colored solution is formed.

Note: The quality of homogenization affects the DNA yield. It is recommended to obtain a homogeneous suspension of the sample.

- Incubate for 10 minutes at 65 °C. Cool the sample to room temperature.

- Homogenization using a mechanical homogenizer

- Take a sample of plant tissues. When working with samples in the RNA stabilizer (ST-100), drain the sample with a clean cloth. Weigh the sample.
- Quickly and carefully, transfer the sample to a clean tube with a screw cap containing 750 ml of buffer for lysis (LB) and, if required, a matrix for homogenization.
- Grind using a mechanical tissue homogenizer, for example, FastPrep-24™ 5G (MP Biomedicals), TissueRuptor II (QIAGEN).

Note: If a homogenization matrix (powder or beads) is used, the quantity should be such that the LB lysis buffer completely covers the homogenization matrix and the tissue sample. If the amount of buffer for lysis (LB) is insufficient, then increase the volume of buffer for lysis (LB) or reduce the amount of matrix for homogenization.

Application to the Column

1. Centrifuge the lysate for 5 min at 12000 rcf. Collect the supernatant and transfer it to a clean test tube.

Note: when the green pigment is extracted from the green parts of plants, it forms a separate upper phase. The collection of the supernatant is carried out without touching the green pigment.

2. Add 5 µl of RNase A. Incubate for 10 minutes at 37° C.
3. Add an equal amount of buffer to be applied to the BB column, mix thoroughly. Incubate for 1 minute at room temperature.
4. Apply no more than 800 ml of lysate to the column. Close the column cover tightly.
5. Centrifuge for 30 s at 12000 rcf. Remove the filtrate.

Note: If a portion of the solution remains on the column after centrifugation, repeat the centrifugation by increasing the time and/or speed of centrifugation, without adding a new portion of the sample or LB buffer.

Note: If the volume of the lysate is more than 800 µl, re-apply the excess to the same column and repeat the centrifugation.

Washing the Column

1. Apply 500 ml of buffer for washing (WB) to the column. Centrifuge for 30 s at 12000 rcf. Remove the filtrate.
2. Re-apply 500 ml of washing buffer to the column. Centrifuge for 30 s at 12000 rcf. Remove the filtrate.

3. Centrifuge the column for 3 min at 12000 rcf to completely remove the WB buffer.

DNA Elution

1. Transfer the column to a new 1.5-2 ml micro test tube (not included in the kit). Press the column firmly against the test tube.
2. Apply 60 to 200 µl of elution buffer (EB) to the filter center of the column. Incubate for 3 minutes at room temperature (15-25° C). Centrifuge for 1 min at 10000 rcf.

- With an increase in the volume of elution, the amount of DNA increases and the concentration of DNA decreases. The amount of DNA during elution with volumes of 60 and 200 µl can vary by 1.5-2 times.
- A second elution with a new aliquot of the elution buffer or repeated application of the eluate to the column allows an additional increase in the amount of DNA.
- The elution buffer contains 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 adjusted with NaOH solution).

3. Eluate containing DNA should be stored at -20° C. For long-term storage, it is recommended to add EDTA (pH 8) to the final concentration of 0.1-1 mM.

Note: EDTA can inhibit enzymatic reactions, for example, PCR.

Analysis of Extracted DNA

The integrity of the extracted DNA can be checked using gel electrophoresis in 1% agarose gel.

The amount of the extracted DNA can be estimated using UV spectrometry.
The characteristic absorption maximum for DNA at λ = 260 nm.
The concentration of DNA (µg/ml) can be calculated using the following formula:
A_{260} * dilution * 50 µg/ml.
Typical ratio of optical densities is A_{260}/A_{280} ~1.7-2.0.

Storage Requirements

The DNA extraction kit can be stored at room temperature (15-25° C). The RNase A solution should be stored at a temperature of -18 to -24° C. See the shelf life on the package.

Transportation Conditions

Transportation of the kit, including the RNase A solution, should be carried out at a temperature of +15 to +25° C. It is allowed to transport the kit at a temperature not higher than +25° C for 14 days.

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sales@biolabmix.ru
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