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EliGene® Plant DNA Isolation Kit
Instructions for Use

Package:

Ref. No.	Quantity
414050P	50 Preps
(Homogenization Pestles Incl.)	
414050	50 Preps
(Homogenization Pestles Not Incl.)	

Storage:

All kit reagents and components should be stored at room temperature (15 – 30 °C). When stored under these conditions, the kit will retain full activity until the expiration date indicated on the kit label.

Table of Contents

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Introduction

EliGene® Plant DNA Isolation Kit is the best choice for fast and easy DNA extraction from plant cells. Using the special homogenization pestle together with efficient inhibitor removal chemistry guarantees high DNA yield from different sample types, including arabidopsis, tobacco, tomato, peach or wild cherry leaves. Manual homogenization of the sample takes just a few seconds at the beginning of isolation. Isolated DNA is ready to be used in any downstream applications including PCR, qPCR and Sanger or NGS sequencing.

Environmental samples are homogenized in tube by mechanical and chemical methods. In the presence of detergent cells are lysed and proteins denatured. In the presence of chaotropic agent DNA is bound to the spin filter, washed and eluted in Tris-HCl buffer without EDTA.

Equipment Required

Microcentrifuge (12,000 x g)
Vortex
Thermostat / Thermoshaker
Microcentrifuge tube rack
Pipettes: 50 – 750 µl
Cold Tube Rack / Ice

Kit Contents

Components	Amount (50 isolations)
Homogenization Sand	13 grams
Homogenization Pestles	50 pcs
Homogenization Buffer P1	24 ml
Lysis Buffer P2	3 ml
Inhibitor Removal Buffer P3	10 ml
Binding Buffer P4	27 ml
Binding Buffer P5	27 ml
Wash Buffer P6	27 ml
Wash Buffer P7	27 ml
Elution Buffer P8	6 ml
1.5 ml Tubes	100 pcs
Spin Filters (Units in 2 ml Collection Tubes)	50 pcs
2 ml Tubes	50 pcs
2 ml Collection Tubes	100 pcs

Precautions

Please wear gloves when using this product. Avoid all skin contact with kit reagents. In case of contact, wash thoroughly with water. Do not ingest. In case of accidental ingestion or contact, see Safety Data Sheets for emergency procedures.
Reagents labelled flammable should be kept away from open flames and sparks.

WARNING: Binding Buffer P5, Wash Buffer P6 and Wash Buffer P7 are flammable.

Recycling of Homogenization Pestles

Incubate the Homogenization Pestles 24 hours in 0.2 M hydrochloric acid solution (HCl). After incubation wash the Homogenization Pestles with sterile distilled water.
If you are not able to prepare 0.2 M hydrochloric acid solution you can contact our Sales Department on info@elisabeth.cz. We can provide it for you.

Detailed Isolation Protocol

It is highly recommended to read this information before you use the EliGene® Plant DNA Isolation Kit for the first time.

Important Notes before Using

Please wear gloves at all times.
If there is precipitate in Lysis Buffer P2, heat the bottle with buffer to 60 °C to dissolve it.
Removal of residual ethanol from the spin filter is critical for efficient elution of DNA from the spin filter by Elution Buffer P8.

1. Add 0.2 grams of Homogenization Sand and 0.05 grams of leaf sample to a 1.5 ml Tube (provided). Use Homogenization Pestle for mashing the leaf and add 450 µl of Homogenization Buffer P1. Briefly vortex.
   Background: Homogenization Buffer P1 contains a solution preventing nucleic acid from degradation and also helps to remove proteins and phenolic compounds.

2. Add 50 µl of Lysis Buffer P2 and mix briefly by vortexing. Incubate for 10 minutes at 70 °C with occasional mixing.
   Background: Lysis Buffer P2 contains SDS. Under low temperature SDS will form a white precipitate in the bottle. Heating to 60 °C will dissolve the SDS again. Lysis Buffer can be used while it is still warm.

3. Centrifuge 1.5 ml Tube at 10,000 x g for 3 minutes at room temperature.
   CAUTION: Be sure not to exceed 10,000 x g or tubes may break.

4. Transfer the supernatant (approximately 450 µl) to a clean 1.5 ml Tube (provided) and add 175 µl of Inhibitor Removal Buffer P3 and vortex for 5 seconds. Incubate on ice or in Cold Tube Rack at -4 °C for 4 – 5 minutes.
   Background: Inhibitor Removal Buffer P3 contains reagents to precipitate non-DNA organic and inorganic material such as proteins, phenolic compounds or polysaccharides.

5. Centrifuge the tube at room temperature for 3 minutes at 10,000 x g.

6. Transfer up to 500 µl of supernatant to a clean 2 ml Tube (provided). Be careful not to disturb the pellet.
   Background: The pellet contains non-DNA organic and inorganic materials. For the best DNA quality avoid disturbing the pellet.

7. Add 500 µl of Binding Buffer P4 and vortex for 5 seconds. Shortly spin to collect the sample from the lid.
   Background: Binding Buffer P4 contains chaotropic salt to provide the optimal conditions for DNA binding but not for non-DNA organic and inorganic material.

8. Add 500 µl Binding Buffer P5 and vortex for 5 seconds. Shortly spin to collect the sample from the lid.
   Background: Binding Buffer P5 contains ethanol to provide the optimal conditions for DNA binding but not for non-DNA organic and inorganic material.

9. Load maximally 750 µl of supernatant onto a spin filter and centrifuge at 10,000 x g for 1 minute at room temperature. Discard the flow through and add an additional 750 µl of supernatant onto the spin filter and centrifuge at 10,000 x g for 1 minute at room temperature.
   Background: DNA binds to the silica membrane in the spin filter because it is in a chaotropic salt condition. The liquid flow through contains unbound cell material.

10. Transfer the spin filter into a new 2 ml Collection Tube (provided).

11. Add 500 µl of Wash Buffer P6 to the spin filter. Centrifuge for 1 minute at 10,000 x g.

Background: Wash Buffer P6 is a salt based wash solution that cleans the DNA bound to the spin filter from other impurities.

12. Remove the spin filter and discard the flow through. Place the spin filter back into the same 2 ml Collection Tube.

13. Add 500 µl of Wash Buffer P7 to the spin filter. Centrifuge for 1 minute at 10,000 x g.
    Background: Wash Buffer P7 is ethanol based wash solution that cleans the DNA bound to the spin filter from the other impurities.

14. Remove the spin filter and discard the flow through. Place the spin filter back into the same 2 ml Collection Tube.

15. Centrifuge again for 2 minutes at 12,000 x g to completely dry the spin filter membrane.
    Background: The spin filter is completely dried from ethanol residues to allow maximal DNA release from the spin filter membrane in elution step.

16. Carefully remove the spin filter and transfer it into a new 2 ml Collection Tube (provided).

17. Add 100 µl of Elution Buffer P8.
    Note: To increase yields, incubate for 5 minutes at 65 °C.

18. Centrifuge 1 minute at 10,000 x g.

19. Remove the spin filter unit. Isolated DNA in the tube is now ready to use in any application.
    Background: Elution Buffer P8 is 10mM Tris-HCl and it releases DNA from the filter and it passes into the 2 ml Collection Tube. The DNA is released due to no salt and no ethanol presence.

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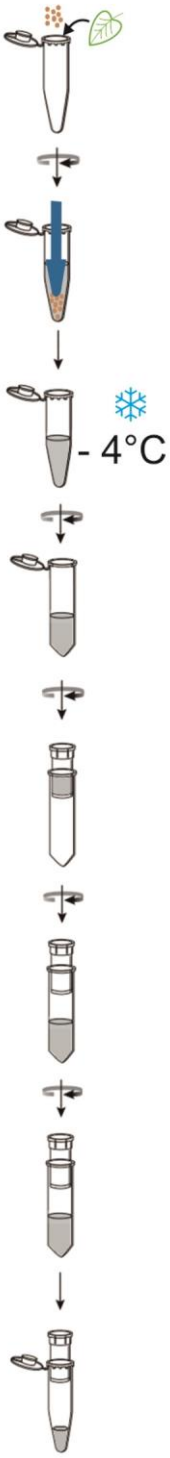
Brief Isolation Protocol



1. Add 0.2 grams of Homogenization Sand and 0.05 grams of leaf sample to a 1.5 ml Tube (provided). Use Homogenization Pestle for mashing the leaf and add 450 µl of Homogenization Buffer P1 and mix.
2. Add 50 µl of Lysis Buffer P2, mix briefly by vortexing and incubate for 10 minutes at 70 °C with occasional mixing.
3. Centrifuge Homogenization Tube at 10,000 x g for 3 minutes at room temperature.
4. Transfer the supernatant to a clean 1.5 ml Tube (provided) and add 175 µl of Inhibitor Removal Buffer P3 and vortex for 5 seconds. Incubate on ice for 4 – 5 minutes.
5. Centrifuge the tube at room temperature for 3 minutes at 10,000 x g.
6. Transfer up to 500 µl of supernatant to a clean 2 ml Tube (provided).
7. Add 500 µl of Binding Buffer P4 and vortex for 5 seconds. Shortly spin.
8. Add 500 µl of Binding Buffer P5 and vortex for 5 seconds. Shortly spin.
9. Transfer 750 µl of supernatant onto a spin filter and centrifuge at 10,000 x g for 1 minute at room temperature. Discard the flow through and add the remaining supernatant (750 µl) onto the spin filter. Each time centrifuge at 10,000 x g for 1 minute at room temperature.
10. Transfer the spin filter into a new 2 ml Collection Tube (provided).
11. Add 500 µl of Wash Buffer P6 to the spin filter. Centrifuge for 1 minute at 10,000 x g.
12. Remove the spin filter and discard the flow through. Place the spin filter back into the same 2 ml Collection Tube.
13. Add 500 µl of Wash Buffer P7 to the spin filter. Centrifuge for 1 minute at 10,000 x g.
14. Remove the spin filter and discard the flow through. Place the spin filter back into the same 2 ml Collection Tube.
15. Centrifuge again for 2 minutes at 12,000 x g to completely dry the spin filter membrane.
16. Carefully remove the spin filter and transfer it into a new 2 ml Collection Tube (provided).
17. Add 100 µl of Elution Buffer P8 and centrifuge 1 minute at 10,000 x g.
18. Remove the spin filter unit. DNA in tube is now ready to use in any application.

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Troubleshooting Guide

Dry Plant Sample

- In case of very low content of water in plant samples start homogenization procedure with 100 µl of Homogenization Buffer P1.
- Make sure that the sample is completely mashed and add 400 µl Homogenization Buffer P1.

If DNA Does Not Amplify

- Make sure to check DNA yields and purity by gel electrophoresis or spectrophotometer reading. An excess amount of DNA would inhibit a PCR reaction.
- Dilute the template DNA.

Eluted DNA Sample Is Green/Brown or Clogging of the Silica Spin Filter

- If you follow recommendations in these instructions, there should not be observed any coloration in isolated DNA.
- Do not use more than 0.05 grams of leaf sample in isolation.

Low DNA Yield

DNA yields may be lower if the leaf has high content of water or has been stored for long time or underwent multiple freeze/thawed cycles. The following points may be checked:

- Make sure to mix the sample well after adding Lysis Buffer P2.
- The temperature for lysis should be set correctly.
- Do not skip the step with removal of residual ethanol from spin filter, it is critical for efficient elution of DNA from the filter by Elution Buffer P8.

DNA has Low A260/280 Ratio

The ratio for pure DNA should be between 1.7 – 1.9. $A_{260/280}$ reading below 1.6 may signify protein contamination. Low $A_{260/280}$ ratios may be caused by the following:

- Make sure to perform the Wash Buffer P6 and Wash Buffer P7 wash to remove proteins.
- If using a Nanodrop, blank the instrument with Elution Buffer P8.

DNA Floats Out of Well When Loaded on a Gel

- The residues of Wash Buffer P7 remain in the final sample. Do not skip the step with removal of residual ethanol from the spin filter. You may extend the dry spin to 2 minutes.

Concentrating the DNA

The final volume of eluted DNA will be 100 µl. The DNA may be concentrated by adding 10 µl of 3 M sodium acetate (pH = 5.2) and inverting 3 – 5 times to mix. Next, add 200 µl of 100% cold ethanol and invert 3 – 5 times to mix and centrifuge at 12,000 x g for 15 minutes at room temperature. Remove supernatant and wash the DNA pellet with 70% ethanol. Evaporate residual ethanol in a speed vac, desiccator, or by ambient air and resuspend DNA in desired volume of PCR water or buffer.

Manufacturer:

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	Catalog number		Upper limit of temperature
	Batch code		Manufacturer
	Use by (last day of month)		Contains sufficient "N" tests