ELISABETH PHARMACON Ltd.
Rokycanova 4437/5
615 00 Brno-Zidenice, Czech Republic
Phone: +420 542 213 851
E-mail: info@elisabeth.cz
Web: www.elisabeth.cz
VAT: CZ26258412

EliGene® Soil DNA Isolation Kit
Instructions For Use

Package:

Ref. No.	Quantity
412050	50 Preps

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

ELISABETH PHARMACON Ltd.
Rokycanova 4437/5
615 00 Brno-Zidenice, Czech Republic
Phone: +420 542 213 851
E-mail: info@elisabeth.cz
Web: www.elisabeth.cz
VAT: CZ26258412

Introduction

EliGene® Soil DNA Isolation Kit is intended for rapid isolation of genomic DNA from environmental samples utilizing our unique technology for the removal of inhibitors. Primary, the isolation kit is designed for use with environmental samples with a high humic acid content, including different soil types such as compost, black soil or manure, and other common soil types. The isolated DNA shows a high purity suitable for successful PCR amplification of organisms from the soil sample. A variety of organisms, including bacteria, fungi, or algae, have been successfully detected from isolated samples by PCR and 16S metagenomics NGS study.

Environmental samples are homogenized in a bead beating 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. DNA is ready to use in PCR, qPCR and sequencing.

Equipment Required

Microcentrifuge (12,000 x *g*)
Vortex
Thermostat/Thermoshaker
Microcentrifuge tube rack
Pipettes: 100 – 700 µl

Kit Contents

Components	Amount (50 isolations)
Homogenization Bead Tubes	50 pcs
Lysis Buffer S1	3,5 ml
Inhibitor Removal Buffer S2	14 ml
Inhibitor Removal Buffer S3	17 ml
Binding Buffer S4	23 ml
Binding Buffer S5	31 ml
Wash Buffer S6	27 ml
Elution Buffer S7	6 ml
Spin Filters (Units in 2 ml Collection Tubes)	50 pcs
2 ml Tubes	150 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. See Safety Data Sheets for emergency procedures in case of accidental ingestion or contact.
Reagents labelled flammable should be kept away from open flames and sparks.

WARNING: Binding Buffer S5 and Wash Buffer S6 are flammable.

Detailed Isolation Protocol

It is highly recommended to read this information before using the EliGene® Soil DNA Isolation Kit for the first time.

Important Notes before Using

Please wear gloves at all times.
If there is precipitate in Lysis Buffer S1, heat the bottle with the buffer to 60 °C to dissolve it.
Removal of residual ethanol from the spin filter is critical for efficient elution of DNA from the filter by Elution Buffer S7.

1. Add 0.2 grams of a soil sample to the Homogenization Bead Tubes (provided) and vortex to mix. In the case of samples with a very high content of humic acid use only 0.1 grams.
   Background: The Homogenization Bead Tubes contain a solution preventing nucleic acids from degradation, and it dissolves humic acids.

2. Add 60 µl of Lysis Buffer S1 and mix briefly by vortexing and incubate for 10 minutes at 70 °C with occasional mixing.
   Background: Lysis Buffer S1 is a lysis reagent containing SDS and other reagents required for complete cell lysis. Under low temperature, SDS will form a white precipitate in the bottle. Heating to 60 °C will dissolve the SDS again. Lysis Buffer S1 can be used while it is still warm.

3. Secure Homogenization Bead Tubes horizontally on a vortex pad with tape and vortex at maximum speed for 10 minutes.

4. Centrifuge Homogenization Bead Tubes at 10,000 x g for 30 seconds at room temperature.
   CAUTION: Be sure not to exceed 10,000 x g or tubes may break.

5. Transfer the supernatant to a clean 2 ml Tube (provided) and add 250 µl of Inhibitor Removal Buffer S2 and vortex for 5 seconds. Incubate on ice for 5 minutes.

Background: Inhibitor Removal Buffer S2 contains a reagent to precipitate non-DNA organic and inorganic material such as humic substances or proteins.

6. Centrifuge the tubes at room temperature for 1 minute at 10,000 x g.

7. Transfer up to 600 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 material such as humic acid or proteins. For the best DNA quality avoid disturbing the pellet.

8. Add 300 µl of Inhibitor Removal Buffer S3 and vortex for 5 seconds. Incubate on ice for 5 minutes.
   Background: Inhibitor Removal Buffer S3 is further precipitating additional non-DNA organic and inorganic material such as humic substances or proteins.

9. Centrifuge the tubes at room temperature for 1 minute at 10,000 x g.

10. Transfer up to 700 µl of supernatant to a clean 2 ml Tube (provided). Be careful not to disturb the pellet.
    Background: The pellet contains additional non-DNA organic and inorganic material such as humic acid or proteins. For the best DNA quality avoid disturbing the pellet.

11. Add 450 µl of Binding Buffer S4 and vortex for 5 seconds. Shortly spin to collect the sample from the lid.
    Background: Binding Buffer S4 contains chaotropic salt to provide optimal DNA binding conditions but not for non-DNA organic and inorganic material.

12. Add 600 µl of Binding Buffer S5 and vortex for 5 seconds. Shortly spin to collect the sample from the lid.
    Background: Binding Buffer S5 contains isopropanol to provide optimal DNA binding conditions but not for non-DNA organic and inorganic material.

13. Load maximally 700 µl 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 700 µl of supernatant onto the spin filter and centrifuge at 10,000 x g for 1 minute at room temperature. Discard the flow-through and load the remaining supernatant (up to 700 µl) onto the spin filter and centrifuge at 10,000 x g for 1 minute at room temperature.
    Background: DNA binds to the spin filter's silica membrane because it is in a chaotropic salt condition. The liquid flow through contains unbound cell material such as denatured proteins and RNA.

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

15. Add 500 µl of Wash Buffer S6 to the spin filter. Centrifuge for 1 minute at 10,000 x g.
Background: Wash buffer S6 is a salt-based wash solution that cleans the DNA bounded to the spin filter from other impurities.

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

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

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

19. Add 100 µl of Elution Buffer S7.
Note: To increase yields, incubate for 5 minutes at 65 °C.

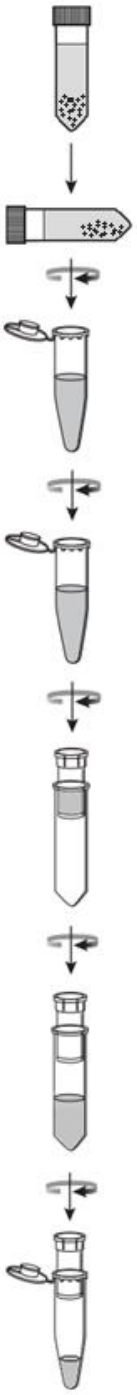
20. Centrifuge 1 minute at 10,000 x g.

21. Remove the spin filter unit. Genomic DNA in the tube is now ready to use in any application.
Background: Elution Buffer S7 is 10 mM Tris, 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.*

ELISABETH PHARMACON Ltd.
Rokycanova 4437/5
615 00 Brno-Zidenice, Czech Republic
Phone: +420 542 213 851
E-mail: info@elisabeth.cz
Web: www.elisabeth.cz
VAT: CZ26258412

Brief Isolation Protocol

1. Add 0.2 grams of a soil sample to the Homogenization Bead Tubes (provided) and vortex to mix.
2. Add 60 µl of Lysis Buffer S1, mix briefly by vortexing and incubate for 10 minutes at 70 °C with occasional mixing.
3. Secure Homogenization Bead Tubes horizontally on a vortex pad with tape and vortex at maximum speed for 10 minutes, centrifuge at 10,000 x g for 30 seconds.
4. Transfer the supernatant to a clean 2 ml Tube (provided) and add 250 µl of Inhibitor Removal Buffer S2 and vortex for 5 seconds. Incubate on ice for 5 minutes.
5. Centrifuge the tube at room temperature for 1 minute at 10,000 x g.
6. Transfer up to 600 µl of supernatant to a clean 2 ml Tube (provided) and add 300 µl of Inhibitor Removal Buffer S3 and vortex for 5 seconds. Incubate on ice for 5 minutes.
7. Centrifuge the tube at room temperature for 1 minute at 10,000 x g.
8. Transfer up to 700 µl of supernatant to a clean 2 ml Tube. Add 450 µl of Binding Buffer S4 and vortex for 5 seconds. Shortly spin to collect the sample from the lid.
9. Add 600 µl of Binding Buffer S5 and vortex for 5 seconds. Shortly spin to collect the sample from the lid.
10. Transfer 700 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 in 700 µl steps onto the spin filter—each time centrifuge at 10,000 x g for 1 minute at room temperature.
11. Transfer spin filter to new 2 ml Collection Tube and add 500 µl of Wash Buffer S6 to 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 and centrifuge again for 1 minute at 12,000 x g.
13. Carefully remove the spin filter and transfer it into a new 2 ml Collection Tube (provided). Add 100 µl of Elution Buffer S7 and centrifuge 1 minute at 10,000 x g.
14. Remove the spin filter unit. Genomic DNA in the tube is now ready to use in any application.



ELISABETH PHARMACON Ltd.
Rokycanova 4437/5
615 00 Brno-Zidenice, Czech Republic
Phone: +420 542 213 851
E-mail: info@elisabeth.cz
Web: www.elisabeth.cz
VAT: CZ26258412

Troubleshooting Guide

Dry Soil Sample

In soil samples with very low content of water start isolation procedure with only 0.1 g of soil sample.

Wet Soil Sample

In the case of soil samples with a very high content of water transfer the content from the homogenization tube to another 1.5 ml tube. Consequently, add to homogenization tube soil sample and centrifuge it for 1 minute at 10,000 x g. Aspirate as much liquid as possible and add content from homogenization tube back to homogenization tube and follow protocol starting at step 2.

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 Brown or Clogging of the Silica Spin Filter

- If you follow recommendations in these instructions, there should not be observed any colouration in isolated DNA.
- Do not use more than 0.2 grams of soil in isolation.

Low DNA Yield

DNA yields may be lower if the soil has a high water content or has been stored for a 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 S1.
- The temperature for lysis should be set correctly.
- Please do not skip the step with removing residual ethanol from the spin filter; it is critical for efficient elution of DNA from the filter by Elution Buffer S7.

DNA has Low A260/280 Ratio

The ratio for pure DNA should be 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 S6 wash to remove proteins.
- If using a Nanodrop, blank the instrument with Elution Buffer S7.

DNA Floats Out of Well When Loaded on a Gel

- The residues of Wash Buffer S6 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 ambient air and resuspend DNA in the desired volume of PCR water or buffer.

Manufacturer:

ELISABETH PHARMACON, spol. s r. o.
Rokycanova 4437/5, Brno-Židenice 615 00
info@elisabeth.cz | www.elisabeth.cz | tel.: +420 542 213 851

	Catalogue number		The upper limit of temperature
	Batch code		Manufacturer
	Use by (last day of the month)		Contains sufficient "N" tests