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® FFPE Tissue DNA Isolation Kit
Instructions for Use

Package:

Ref. No.	Quantity
415050	50 Preps

Storage:

All kit reagents and components should be stored at room temperature (15 – 30 °C). After delivery Proteinase K solution must be stored at 2 - 8 °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® FFPE Tissue DNA Isolation Kit is intended for isolation of genomic DNA from paraffin embedded and formalin fixed tissues. The tissue is released by complete dissolution of the wax by buffer. 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. There is no need to use xylene or additional ethanol for wax removal. Everything is included in the kit.

Incubation at 90 °C partially removes formaldehyde crosslinking of the released DNA, improving yields and DNA amplification. Quality and yield of isolated DNA depends on the quality of FFPE tissue, the tissue source, the age of the sample, thickness of the slice and the amount of wax in each slice. Measuring DNA concentration depends on the chosen method, e.g. spectrophotometry measures both double-stranded and single-stranded DNA while fluorometry with PicoGreen® (Molecular Probes, Inc.) measures only double-stranded DNA. DNA isolated from FFPE tissue is more fragmented than from fresh or frozen tissue. This leads to lower molecular weight and smearing on a gel. Older samples and samples of lower quality lead to greater smear, thus it is recommended to amplify shorter DNA regions by PCR.

Equipment Required

Microcentrifuge (12,000 x g)
Vortex
Thermostat/Thermoshaker capable of incubation at 90 °C
Pipettes: 50 – 750 µl

Kit Contents

Components	Amount (50 isolations)
Lysis Buffer FT1	11 ml
Binding Buffer FT2	12 ml
Binding Buffer FT3	12 ml
Wash Buffer FT4	27 ml
Wash Buffer FT5	27 ml
Elution Buffer FT6	6 ml
Proteinase K (20 mg/ml)	1 ml
1.5 ml Tubes	100 pcs
Spin Filters (Units in 2 ml Collection Tubes)	50 pcs
2 ml Collection Tubes	50 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 FT3, Wash Buffer FT4 and Wash Buffer FT5 are flammable.

Detailed Isolation Protocol

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

Important Notes Before Using

- **Please wear gloves at all times.**
- **If there is precipitate in Lysis Buffer FT1 or/and in Binding Buffer FT2, 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 filter by Elution Buffer FT6.**
- **! Read steps 3 and 4 before starting the DNA isolation (First, heat thermomixer to 56 °C for 1 hour incubation. Secondly, heat it to 90 °C for 1 hour incubation.)!**

1. Add 1 or more FFPE tissue slices to the 1.5 ml tube (do not use more than 20 mg). If the sample surface has been exposed to air, discard the first 2 sections for better quality of isolated DNA.
2. Add 210 µl of Lysis Buffer FT1 and 20 µl of Proteinase K solution. Mix the sample by vortexing.
   Background: Proteinase K breaks down the cell wall and helps lyse the cells. No incubation isnecessary.
3. Transfer the sample to the thermomixer and incubate at 56 °C for 1 hour.
4. Incubate at 90 °C for 1 hour.
   Background: If using only one heating block, leave the sample at room temperature after the 56 °C and wait until the block has reached 90 °C.
5. Centrifuge the sample at 12 000 x g for 1 minute. Transfer the 200 µl lysate to a new 1.5 ml tube.

Background: Cooling creates a tough layer of wax on the surface of the lysate. Carefully remove the lysate without transferring the wax.

6. Add 220 µl of Binding Buffer FT2 and mix by vortexing, shortly centrifuge.
   Background: Binding Buffer FT2 contains chaotropic salt to provide the optimal conditions for DNA binding but not for non-DNA organic and inorganic material.
7. Add 220 µl of Binding Buffer FT3 and mix by vortexing, shortly centrifuge.
   Background: Binding Buffer FT3 contains chaotropic salt to provide the optimal conditions for DNA binding but not for non-DNA organic and inorganic material.
8. Load the lysate onto a spin filter and centrifuge at 8 000 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.
9. Remove the spin filter and discard the flow through. Place the spin filter back into the same 2 ml Collection Tube.
10. Add 500 µl of Wash Buffer FT4 to the spin filter. Centrifuge for 1 minute at 8 000 x g.
    Background: Wash Buffer FT4 is a salt based wash solution that cleans the DNA bound to the Spin Filter from other impurities.
11. Remove the spin filter and discard the flow through. Place the spin filter back into the same 2 ml Collection Tube.
12. Add 500 µl of Wash Buffer FT5 to the spin filter. Centrifuge for 1 minute at 8 000 x g.
    Background: Wash Buffer FT5 is a salt based wash solution that cleans the DNA bound to the Spin Filter from other impurities.
13. Remove the spin filter and discard the flow through. Place the spin filter back into the same 2 ml Collection Tube.
14. 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 for maximal DNA release from the Spin Filter membrane in elution step.
15. Carefully remove the spin filter and transfer it into a new 2 ml Collection Tube (provided).
16. Add 50 - 100 µl of Elution Buffer FT6.

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

17. Incubate at room temperature for 1 minute. Centrifuge 1 minute at 10 000 x g.

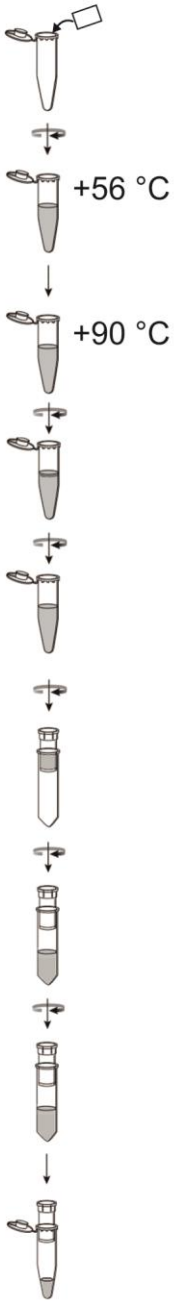
    Note: To increase yields, incubate for 5 minutes at 65 °C.

18. Remove the spin filter unit. DNA in 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

Brief Isolation Protocol

1. Add 1 or more FFPE tissue slices to the 1.5 ml tube (do not use more than 20 mg).
2. Add 210 µl of Lysis Buffer FT1 and 20 µl of Proteinase K solution. Mix the sample by vortexing.
3. Transfer the sample to the thermomixer and incubate at 56 °C for 1 hour.
4. Incubate at 90 °C for 1 hour.
5. Centrifuge the sample at 12 000 x g for 1 minute. Transfer the lysate (200 µl) to a new 1.5 ml tube.
6. Add 220 µl of Binding Buffer FT2 and mix by vortexing, shortly centrifuge.
7. Add 220 µl of Binding Buffer FT3 and mix by vortexing, shortly centrifuge
8. Load the lysate onto a spin filter and centrifuge at 8 000 x g for 1 minute at room temperature.
9. Remove the spin filter and discard the flow through. Place the spin filter back into the same 2 ml Collection Tube.
10. Add 500 µl of Wash Buffer FT4 to the spin filter. Centrifuge for 1 minute at 8 000 x g.
11. Remove the spin filter and discard the flow through. Place the spin filter back into the same 2 ml Collection Tube.
12. Add 500 µl of Wash Buffer FT5 to the spin filter. Centrifuge for 1 minute at 8 000 x g.
13. Remove the spin filter and discard the flow through. Place the spin filter back into the same 2 ml Collection Tube.
14. Centrifuge again for 2 minutes at 12 000 x g to completely dry the spin filter membrane.
15. Carefully remove the spin filter and transfer it into a new 2 ml Collection Tube (provided).
16. Add 50 - 100 µl of Elution Buffer FT6.
17. Incubate at room temperature for 1 minute. 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.



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

If DNA Does Not Amplify

- Make sure to check DNA yields and purity by gel electrophoresis, UV spectrophotometer or fluorometer (using PicoGreen®, Molecular Probes, Inc.) reading. An excess amount of DNA would inhibit a PCR reaction.
- Make sure to mix properly Wash Buffers FT4 and FT5 after being unused for a longer time. Components of the buffers may have separated out.
- Dilute the template DNA.

Eluted DNA Sample Is Colored 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 20 mg of FFPE tissue in isolation.

Low DNA Yield

- Please note that DNA yields and quality will vary and will be lower compared to fresh or frozen tissue. The quality and concentration of the DNA isolate is influenced by the quality of FFPE tissue, the tissue source, the age of the sample, thickness of the slice and the amount of wax in each slice.
- Take into consideration that different measuring methods result in different concentrations depending whether UV spectrometry of fluorometry is used. Spectrophotometry measures both double-stranded and single-stranded DNA while fluorometry with PicoGreen® (Molecular Probes, Inc.) measures only double-stranded DNA**.**
- Make sure to mix the sample well after adding Lysis Buffer FT1.
- The temperature for lysis should be set correctly.
- In order to maintain DNA integrity, make sure not to exceed 90 °C.
- 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 FT6.

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 FT5 wash to remove proteins.
- If using a Nanodrop, blank the instrument with Elution Buffer FT6.

DNA Floats Out of Well When Loaded on a Gel

- The residues of Wash Buffer FT5 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 3 minutes.

Concentrating the DNA

The final volume of eluted DNA will be 50 - 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 cold 100% ethanol and invert 3 – 5 times to mix and centrifuge at 12,000 x g for 15 minutes at room temperature. Remove the 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:

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

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