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# EliGene® Tissue DNA Isolation Kit
# Instructions for Use

## *Package:*

| Ref. No. | Quantity |
|---|---|
| 413050P | 50 Preps |
| (Homogenization Pestles Incl.) | |
| 413050 | 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*

The EliGene® Tissue DNA Isolation Kit is designed for isolation of genomic DNA from tissues. 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.

The quality and yield of isolated DNA depends on the tissue quality, the tissue source, the age of the sample and the tissue amount. Measurement of DNA concentration depends on the chosen method, e.g. spectrophotometry measures both double-stranded and single-stranded DNA, whereas PicoGreen® fluorimetry (Molecular Probes, Inc.) measures only double-stranded DNA. DNA isolated from frozen tissue is fragmented more than fresh tissue, which can lead to blurred stripes on the gel. Older samples and lower quality samples also lead to more blurred stripes on the gel. In these cases, it is recommended to amplify shorter DNA regions by PCR.

## *Equipment Required*

Mikrocentrifuge (12,000 x g)
Vortex
Thermostat/Thermoshaker, incubation at 65 °C
Pipettes: 50 – 750 µl
Weighing scales

## *Kit Contents*

| Components | Amount (50 isolations) |
|---|---|
| Lysis Buffer T1 | 16.5 ml |
| Binding Buffer T2 | 14 ml |
| Binding Buffer T3 | 14 ml |
| Wash Buffer T4 | 27 ml |
| Wash Buffer T5 | 27 ml |
| Elution Buffer T6 | 6 ml |
| Homogenization Pestles (413050P) | 50 pcs |
| Homogenization Sand | 13 g |
| 1.5 ml Tubes | 50 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. In case of contact or accidental ingestion, see Safety Data Sheets for emergency procedures.
Reagents labelled flammable should be kept away from open flames and sparks.

**WARNING:** Binding Buffer T3, Wash Buffer T4 and Wash Buffer T5 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® 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 T1 and/or in Binding Buffer T2, 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 T6.**

1. Add 0.2 g of Homogenization Sand, 15 – 20 mg of the tissue sample and 50 µl of Lysis Buffer T1 to 1.5 ml tube (provided). Use Homogenization Pestle for mashing the sample.
2. Add 250 µl of Lysis Buffer T1 and mix briefly by vortexing.

   *Background: Lysis Buffer T1 includes a solution preventing the degradation of nucleic acids and helps removing the proteins. Lysis Buffer T1 includes all components necessary for the complete lysis of cells. The buffer can be used while it is still warm.*
3. Transfer the sample into thermostat. Incubate for 20 minutes at 65 ° C.
4. Add 250 µl of Binding Buffer T2 and vortex. Shortly spin to collect the sample from the lid.

*Background: Binding Buffer T2 contains chaotropic salt to provide the optimal conditions for DNA binding but not for non-DNA organic and inorganic material.*

5. Add 250 µl of Binding Buffer T3 and vortex.

   *Background: Binding Buffer T3 contains ethanol to provide the optimal conditions for DNA binding but not for non-DNA organic and inorganic material.*

6. Centrifuge the sample at 10,000 x g for 2 minutes.
7. Transfer the supernatant onto the 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.*

8. Remove the spin filter and discard the flow through. Place the spin filter back into the same 2 ml Collection Tube.
9. Add 500 µl of Wash Buffer T4 to the spin filter. Centrifuge at 8,000 x g for 1 minute.

   *Background: Wash Buffer T4 is ethanol based wash solution that cleans the DNA bound to the spin filter from other impurities.*

10. Remove the spin filter and discard the flow through. Place the spin filter back into the same 2 ml Collection Tube.
11. Add 500 µl of Wash Buffer T5 to the spin filter. Centrifuge at 8,000 x g for 1 minute. Remove the spin filter and discard the flow through. Place the spin filter back into the same 2 ml Collection Tube.

    *Background: Wash Buffer T5 is ethanol based wash solution that cleans the DNA bound to the spin filter from the other impurities.*

12. Centrifuge at 12,000 x g for 2 minutes 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 the elution step.*

13. Carefully remove the spin filter and transfer it into a new 2 ml Collection Tube (provided).
14. Add 50 – 100 µl of Elution Buffer T6.

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

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

    *Background: Elution Buffer T6 is 10 mM Tris-HCl. 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.*

16. Remove the spin filter unit. DNA in the tube is now ready to use in any application.

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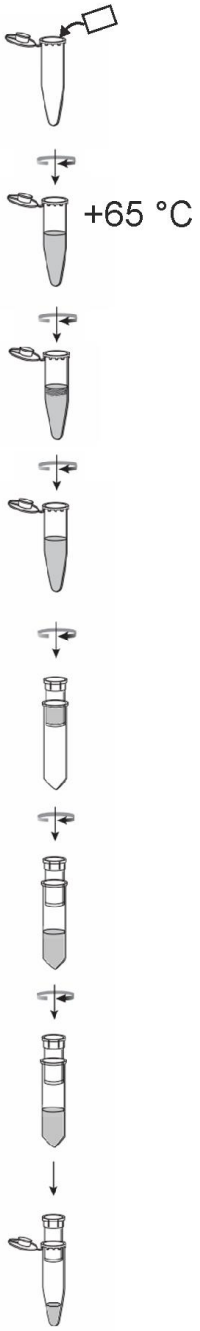
## *Brief Isolation Protocol*




1. Add 0.2 grams of Homogenization Sand, 50 µl of Lysis Buffer T1 and 15 – 20 mg of the tissue sample to a 1.5 ml Tube (provided). Use Homogenization Pestle for mashing the sample.
2. Add 250 µl of Lysis Buffer T1 and briefly vortex.
3. Transfer the sample to thermostat and incubate for 20 minutes at 65 ° C.
4. Add 250 µl of Binding Buffer T2 and vortex, shortly spin.
5. Add 250 µl of Binding Buffer T3 and vortex.
6. Centrifuge the sample at 10,000 x g for 2 minutes.
7. Transfer the supernatant onto the spin filter and centrifuge at 8,000 x g for 1 minute at room temperature.
8. Remove the spin filter and discard the flow through. Place the spin filter back into the same 2 ml Collection Tube.
9. Add 500 µl of Wash Buffer T4 to the spin filter. Centrifuge at 8,000 x g for 1 minute.
10. Remove the spin filter and discard the flow through. Place the spin filter back into the same 2 ml Collection Tube.
11. Add 500 µl of Wash Buffer T5 to the spin filter. Centrifuge at 8,000 x g for 1 minute. Remove the spin filter and discard the flow through. Place the spin filter back into the same 2 ml Collection Tube.
12. Centrifuge at 12,000 x g for 2 minutes to completely dry the spin filter membrane.
13. Carefully remove the spin filter and transfer it into a new 2 ml Collection Tube (provided).
14. Add 50 – 100 µl of Elution Buffer T6.
15. Incubate for 1 minute at room temperature. Centrifuge at 10,000 x g for 1 minute.
16. Remove the spin filter unit. DNA in the tube is now ready to use in any application.

## *Troubleshooting Guide*

### If DNA Does Not Amplify

- Make sure to check DNA yields and purity by gel electrophoresis, UV spectrophotometer or (fluorimeter PicoGreen®, Molecular Probes, Inc.) reading. An excess amount of DNA would inhibit the PCR reaction.
- Make sure to mix Wash Buffer T4 and T5 if you did not use them for a longer period of time. Components may have separated.
- 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.
- If the spin column is clogged, increase the volume of the T2 and T3 solution to 300 µl. If the entire volume will not flow through after step no. 7 and a small layer of lysate will remain above the filter, add T4 solution and continue according to the instructions. If the entire volume of the spin column flows through, the insolation is alright. If the T4 solution does not flow through, it is necessary to repeat the isolation with a smaller weighing of the tissue.
- Do not use more than 20 mg of tissue.

### Low DNA Yield

- Please note that DNA yields and quality will vary when comparing fresh to frozen tissue. The quality and concentration of the DNA isolate is influenced by the quality of tissue, the tissue source, the age of the sample and the amount of tissue.
- 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 T1.
- 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 T6.

### 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 T5 wash to remove proteins.
- If using a Nanodrop, blank the instrument with Elution Buffer T6.

## DNA Floats Out of Well When Loaded on a Gel

- The residues of Wash Buffer T5 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 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 |