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EliGene® Blood DNA Isolation Kit

Instructions For Use

Package:

Ref. no.	Quantity
411050	50 Preps

Storage:

All kit reagents and components of the kit must be transported 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

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Introduction

EliGene® Blood DNA Isolation Kit is intended for rapid isolation of genomic and mitochondrial DNA from whole human blood (fresh, frozen or stored at 4 °C) and buffy coat. In the presence of detergent and Proteinase K 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: 20 – 700 µl

Kit Contents

Components	Amount (50 isolations)
Lysis Buffer B1	12 ml
Binding Buffer B2	12 ml
Wash Buffer B3	27 ml
Wash Buffer B4	27 ml
Elution Buffer B5	12 ml
Proteinase K (20 mg/ml)	1 ml
Spin Filters (Units in 2 ml Tubes)	50 pcs
2 ml Collection Tubes	100 pcs
1.5 ml Lysis 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 B2, Wash Buffer B3 and Wash Buffer B4 are flammable.

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Detailed Isolation Protocol

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

Important Notes Before Using

Please wear gloves at all times.
If there is precipitate in Lysis Buffer B1, heat the bottle with buffer to 50 °C to dissolve it.
Removal of residual ethanol from Spin Filter is critical for efficient elution of DNA from the filter by Elution Buffer B5.

1. Add 200 µl of whole blood to a 1.5 ml Lysis Tube (provided) and add 20 µl of Proteinase K.
   Background: Proteinase K breaks down the cell wall and helps lyse the cells. No incubation is necessary.

2. Add 200 µl of Lysis Buffer B1 and mix by vortexing for 15 seconds.
   Background: Lysis Buffer B1 is a lysis reagent containing a chaotropic salt and non-ionic detergent.

3. Incubate the sample at 65 °C for 15 minutes and shortly spin to collect the lysate from the lid.
   Background: Heat helps denature proteins and completes lysis.

4. Add 210 µl of Binding Buffer B2 and vortex briefly for 15 seconds. Shortly spin to collect the sample from the lid and incubate sample 2 minutes at room temperature.
   Background: Binding Buffer B2 contains propanol and chaotropic salt to provide optimal conditions for DNA binding.

5. Transfer the whole lysate (630 µl) to the Spin Filter and centrifuge for 1 minute at 10,000 x g.
   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 such as denatured proteins and RNA.

6. Transfer Spin Filter to a new 2 ml Collection Tube (provided).

7. Add 500 µl of Wash Buffer B3 to Spin Filter. Centrifuge for 1 minute at 10,000 x g.
   Background: Wash Buffer B3 is a salt based wash solution that cleans the DNA bound to the Spin Filter from other impurities.

8. Remove the Spin Filter and discard the flow through. Place Spin Filter back into the same 2 ml Collection Tube.

9. Add 500 µl of Wash Buffer B4 to Spin Filter. Centrifuge 1 minute at 10,000 x g.

Background: Wash Buffer B4 is an ethanol based wash solution that cleans the DNA bound to the Spin Filter. It removes all residual salt contaminants.

10. Remove the Spin Filter and discard the flow through. Place the Spin Filter back into the same 2 ml Collection Tube.

11. Centrifuge again for 2 minutes at 12,000 x g to 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.

12. Carefully remove Spin Filter and transfer it to a new 2 ml Collection Tube (provided).

13. Add 100 µl of Elution Buffer B5.
    Note: To increase yields, incubate for 5 minutes at 65 °C.

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

15. Remove Spin Filter unit. Genomic DNA in tube is now ready to use for any application.
    Background: Elution Buffer B5 is 10mM 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 ethanol presence.

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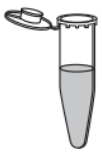
Brief Isolation Protocol

1. Add 200 µl of whole blood to a 1.5 ml Lysis Tube (provided) and add 20 µl of Proteinase K.
2. Add 200 µl of Lysis Buffer B1 and mix by vortexing for 15 seconds.
3. Incubate the sample at 65 °C for 15 minutes and shortly spin to collect the lysate from the lid.
4. Add 210 µl of Binding Buffer B2 and vortex for 15 seconds. Shortly spin to collect the sample from the lid.
5. Transfer the whole lysate (630 µl) to the Spin Filter and centrifuge for 1 minute at 10,000 x g.
6. Transfer Spin Filter to a new 2 ml Collection Tube (provided).
7. Add 500 µl of Wash Buffer B3 to Spin Filter. Centrifuge for 1 minute at 10,000 x g.
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 B4 to Spin Filter. Centrifuge 1 minute at 10,000 x g.
10. Remove the Spin Filter and discard the flow through. Place the Spin Filter back into the same 2 ml Collection Tube.
11. Centrifuge again for 2 minutes at 12,000 x g to dry the Spin Filter membrane.
12. Carefully remove Spin Filter and transfer to a new 2 ml Collection Tube (provided).
13. Add 100 µl of Elution Buffer B5. Centrifuge 1 minute at 10,000 x g
14. Remove the Spin Filter unit. Genomic DNA in tube is now ready to use for any application.



To 200 µl of Blood add:
200 µl of Lysis Buffer B1
20 µl of Proteinase K.
Incubate 15 min at 65 °C.

Add 210 µl of Binding Buffer B2. Transfer to spin filter column.

Wash with 500 µl of Wash Buffer B3.

Wash with 500 µl of Wash Buffer B4.

Elute DNA to 100 µl of Elution Buffer B5.

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Troubleshooting Guide

Clogging of the Silica Spin Filter

If you follow recommendation in these instructions, the silica membrane should not clog. The clogging of Spin Filters could occur from the following reasons:

- Incomplete lysis. Make sure to mix well after adding Lysis Buffer B1 and ensure that the Proteinase K Solution was added. The temperature for lysis should be set correctly.
- Do not use more than 200 µl of human blood. For other animal species, start with 50 – 100 µl of blood and dilute the blood sample to 200 µl by PBS buffer.
- Blood must be treated with anti-coagulants before processing to avoid blood clots. Do not use with clotted blood. If you observed blood clots, remove the clots and use only the liquid part of the sample.

Low DNA Yield

The average yield for 200 µl of human blood is about 2 – 4 µg of DNA. DNA yields may be lower if the sample has been stored for long time or repeatedly frozen/thawed. The following points may be checked:

- Incomplete lysis. Make sure to mix the sample well after adding Lysis Buffer B1 and ensure that the Proteinase K Solution was added.
- The temperature for lysis should be set correctly.
- Do not pre-mix the Proteinase K Solution and the Lysis Buffer B1 before using.
- 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 5.

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 B3 wash before the Wash Buffer B4 wash to remove proteins.
- If using a Nanodrop, blank the instrument with Elution Buffer B5.

DNA Floats Out of Well When Loaded on a Gel

- The residues of Wash Buffer B4 remain in the final sample. Do not skip the step with removal of residual ethanol from Spin Filter and you may extend the dry spin to 1 – 2 minutes.

Concentrating the DNA

The final volume of eluted DNA will be 100 µl. The DNA may be concentrated by adding 4 µl of 5 M NaCl and inverting 3 – 5 times to mix. Next, add 400 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.

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Catalog number

Batch code


Use by (last day of month)


Upper limit of temperature


Manufacturer

Contains sufficient "N" tests