

mi-Blood Genomic DNA Isolation Kit

For highly pure and rapid purification of genomic DNA from whole blood or other body fluids

Cat. No mi-BG100
[100 Preparations]

This kit is for research purposes only.
Not for use in diagnostic procedures.
For in vitro use only.

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Introduction

The mi-Blood Genomic DNA Isolation kit is designed for rapid, simultaneous preparation of highly pure genomic DNA from whole blood, serum plasma, or other body fluids. The obtained DNA can be used directly for PCR, Southern Blotting, or any kind of enzymatic reaction.

The kit allows purification of up to 10 µg of pure genomic DNA from 200 µl whole blood with an A260/280 ratio between 1.6 and 1.9 and a typical concentration of 20-100 ng per µl. Blood treated either with EDTA, citrate, or heparin can be used. The procedure is optimized for a sample volume of 200 µl of samples diluted in PBS buffer.

Kit Contents

	mi-Blood Genomic DNA Isolation Kit
Preparation	100 rxn/kit
Nuclei Lysis Buffer	25 ml
DNA Binding Buffer	27.5 ml (NOTE: Add 27.5 ml of pure ethanol (99.9%) up to a final volume of 55 ml prior to first use.)
Column Wash Buffer	55 ml (NOTE: Add 55 ml of pure ethanol (99.9%) up to a final volume of 110 ml prior to first use).
Proteinase K	store powder at -20°C
Proteinase K storage buffer	store at -20°C
Spin filter	100
Collection tube	200

Required Equipment

Microcentrifuge (13,000 rpm or 12,000 x g)
Vortexer
Microcentrifuge tubes
Heating block at 56°C
Ethanol for the DNA Binding Buffer and the Column Wash Buffer
PBS if cells are contained in <200µl
Tris Buffer (10 mM Tris-HCl, pH 8.0)
Distilled water (pH 7-8) or 10 mM Tris-HCl (pH 8.0)

Kit Storage

Store Proteinase K and the buffer at -20°C upon kit arrival. Store solvated Proteinase K at -20°C. All other kit components are stable at room temperature for 6 months.

Precautions

See MSDS on our homepage (www.mymetabion.com).

Nuclei Lysis buffer and DNA binding buffer contain chaotropic salt!
Wear gloves and goggles!

The basic principle

With the mi-Genomic DNA Isolation method, genomic DNA is prepared from blood cells. The lysis is achieved by the incubation of whole blood in a solution containing large amounts of chaotropic ions in the presence of Proteinase K at 56°C. Appropriate conditions for the binding of DNA to the specialized silica-based membrane in the spin columns are created by the addition of ethanol to the cell lysate. The binding process is reversible and specific to nucleic acids. Contaminations are removed by washing with ethanol-based wash buffer. Pure genomic DNA is finally eluted with Tris buffer (10 mM Tris-HCl, pH 8.0) or distilled water (pH 7–8).

Note: Before starting, please make sure to:

- To add 27.5 ml of pure ethanol (99.9%) to the DNA Binding Buffer prior to first use.
- To add 55 ml of pure ethanol (99.9%) to the Column Wash Buffer prior to first use.
- Before first use of the kit solve the Proteinase K in 1100 µl of the Proteinase K buffer (contained in kit).

Divide the stock solution (20mg/ml) into small aliquots and store them at -20°C.

- Upon storage, especially at low temperature, a white precipitate may form in the Nuclei Lysis Buffer or DNA binding buffer. Dissolve such precipitates by the incubation of the bottle for a few minutes at 50°C before use.
- Before starting the preparation, set incubator, water bath, or oven to 56°C.

Protocol for purification of DNA from whole blood or other body fluids

1. Pipette 10 µl of the Proteinase K solution to the bottom of each 1.5 ml tube.
2. Add 200 µl of the blood sample to a microcentrifuge tube.
Use up to 200 µl whole blood, plasma, buffy coat, or other body fluids.
For small sample volumes, use 1x PBS buffer to adjust to 200 µl.
3. Add 200 µl of Nuclei Lysis Buffer to the sample, mix by pulse-vortexing for 15 sec.
In order to ensure efficient lysis, it is essential that the sample and the Nuclei Lysis Buffer are mixed thoroughly to yield a homogeneous solution.
Note: Do not add Proteinase K directly to the Nuclei Lysis Buffer.
4. Incubate at 56°C for 10 min.
DNA yield reaches a maximum after 10 min at 56°C. Longer incubation times have effect on yield or quality of the purified DNA.
5. Briefly spin the 1.5 ml microcentrifuge tube to remove drops from the inside of lid.
6. Add 200 µl of ethanol (99,9%) to the sample, and mix again by pulse-vortexing for 15 sec.

7. Spin the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid. Put a spin column in a 2 ml collection tube in the meantime.
8. Add 500 μ l of DNA Binding Buffer without wetting the rim and mix by vortexing. (Make sure to have completed the "DNA Binding Buffer" by adding 27.5 ml of pure ethanol before first use).
Add 650 μ l of the sample mix to a spin column.
9. Spin at 13,000 rpm (12,000 x g) for 1 min. The liquid will flow through the spin column membrane leaving the genomic DNA bound to the filter membrane.
Remove the spin column from the collection tube and discard the flow through. Place the spin column in the same sample collection tube. Add the residual mixture (prepared in step 8) to the spin column and spin at 13,000 rpm (12,000 x g) for 2 min. Remove the spin column from the collection tube and discard the flow through.
- 10a. Add 500 μ l of Column Wash Buffer without wetting the rim. (Make sure to have completed the the "Column Wash Buffer" by adding 55 ml of pure ethanol before first use.)
Spin at 13,000 rpm (12,000 x g) for 1 min.
Discard the filtrated liquid and spin at full speed (13,000 rpm or 12,000 x g) for 3 min.
Continue directly with step 11, or to eliminate any possibility of washing buffer carryover, perform step 10b., and then continue on to step 11.
- 10b. (Optional) Replace the spin column in the emptied 2 ml collection tube. Spin again at full speed (13,000 rpm or 12,000 x g) for 1 min.
11. Place the spin column in a clean 1.5 ml microcentrifuge tube (not included), and discard the collection tube containing the filtrate.
Add 100 - 200 μ l Tris buffer (10 mM Tris-HCl, pH 8.0, not included) or distilled water (pH7-8).
Incubate the spin column at room temperature (15-25°C) for 1 min, and spin at 13,000 rpm (12,000 x g) for 1 min.

Incubating the spin column loaded with Tris buffer (10 mM Tris-HCl, pH 8.0) or distilled water for 5 min at room temperature before the centrifugation generally increases DNA yield.

The elution efficiency will decrease when using elution buffers with pH <7.0.

Hints and Troubleshooting

1) No or poor DNA yield

Low concentration of leukocytes in sample

Prepare buffy coat from the blood sample: Spin the whole blood at room temperature at 3,300 x g for 10 min. Three different layers will be visible after the centrifugation. Leukocytes are concentrated in the intermediate layer (=buffy coat).

Incomplete cell lysis

The sample was not thoroughly mixed with Nuclei Lysis Buffer / Proteinase K. The mix has to be vortexed vigorously immediately after addition of Nuclei Lysis Buffer.

- Proteinase K digestion was not optimal. Never add the protease directly to the Nuclei Lysis Buffer. Incubate for 10 min at 56°C.

Suboptimal elution of DNA from the spin column

-Preheat Tris buffer or distilled water to 70°C before elution. Apply Tris buffer or distilled water directly onto the center of silica membrane.

2) Poor DNA quality

Incomplete cell lysis

The sample was not thoroughly mixed with Nuclei Lysis Buffer / Proteinase K. The mix has to be vortexed vigorously immediately after addition of Nuclei Lysis Buffer.

RNA in sample

If DNA free of RNA is desired, add 20 µl of RNase A solution (stock solution 20 mg/mL) before the addition of Nuclei Lysis Buffer.

Old or clotted blood samples processed

For isolation of DNA from older or clotted blood samples, we recommend the extension of protease incubation to 30 min. Vortex once or twice during the incubation.