

Blood Genomic DNA Isolation Mini Kit

Product # 46300

Please note that a more detailed protocol is available online at www.norgenbiotech.com

Component	Product # 46300 (50 samples)
Lysis Solution	20 mL
Wash Solution I	18 mL
Wash Solution II	18 mL
Elution Buffer	12 mL
Proteinase K	1.2 mL
Spin Columns	50
Collection Tubes	50
Elution Tubes	50
Product Insert	1

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 1 year in their unopened containers. The kit contains a ready-to-use Proteinase K solution, which is dissolved in a specially prepared storage buffer. The Proteinase K is stable for up to 1 year after delivery when stored at room temperature. To prolong the lifetime of Proteinase K, storage at 2–8°C is recommended.

Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for human or diagnostic use. Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. The **Lysis Solution** and **Wash Solution I** contain guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions. Blood of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with blood.

Procedures

Notes Prior to Use:

- Ensure that all solutions are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- For best results, the use of whole blood collected into tubes containing an anticoagulant is highly recommended.
- Both fresh and frozen anticoagulated blood may be used with this procedure. Ensure that frozen blood is thawed at room temperature prior to starting the protocol.
- Prepare a working concentration of the **Wash Solution I** by adding 24 mL of 96 - 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution I**. This will give a final volume of 42 mL.
- Prepare a working concentration of the **Wash Solution II** by adding 42 mL of 96 - 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution II**. This will give a final volume of 60 mL.
- **Always** vortex the Proteinase K before use.

1. Sample Preparation

- Add 20 µL of **Proteinase K** (vortex before use) to a microcentrifuge tube.
- Transfer 20 - 200 µL of blood sample to the tube containing **Proteinase K**.
- Add 300 µL of **Lysis Solution** to the blood and mix well by vortexing for 10 seconds.
- Briefly spin the tube to collect any drops of liquid from the inside of the lid.
- Incubate at 55°C for 10 minutes.

Note: If any debris is present in the sample, centrifuge for 2 minutes at 14,000 x g (~14,000 RPM) to precipitate. Transfer the clean supernatant to a microcentrifuge tube prior to **Step f**.

- Briefly spin the tube to collect any drops of liquid from the inside of the lid.
- Add 250 µL of 96-100% Ethanol to the sample and mix well by vortexing for 10 seconds.
- Briefly spin the tube to collect any drops of liquid from the inside of the lid.

2. Sample Binding to Column

- a. Assemble a column with one of the provided collection tubes.
- b. Apply the lysate to the column and centrifuge for 1 minute at 6,000 x g (~8,000 RPM).
- c. Discard the flowthrough. Reassemble the column and the collection tube.

Note: Ensure that all of the lysate has passed through into the collection tube. If the entire lysate volume has not passed, centrifuge for an additional 2 minutes.

3. Column Wash

- a. Apply 500 µL of **Wash Solution I** (ensure ethanol was added) to the column and centrifuge for 1 minute at 6,000 x g (~8,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.

Note: Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

- b. Apply 500 µL of **Wash Solution II** (ensure ethanol was added) to the column and centrifuge for 1 minute at 14,000 x g (~14,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.
- c. Wash column another time by adding 500 µL of **Wash Solution II** and centrifuging for 1 minute at 14,000 x g (~14,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.
- d. Spin the column for 2 minutes in order to thoroughly dry the column at 14,000 x g (~14,000 RPM). Discard the collection tube.

4. DNA Elution

- a. Place the column into a provided 1.7 mL elution tube.
- b. Add 200 µL of **Elution Buffer** to the column.
- c. Incubate at room temperature for 1 minute.
- d. Centrifuge for 1 minute at 6,000 x g (~8,000 RPM)

(Optional): An additional elution may be performed if desired by repeating steps **4a – 4c**. Collect second elution into a new microcentrifuge tube. The yield can be improved by an additional 20-30% when this second elution is performed.

Note: A smaller elution volume (down to 50 µL) can be used to obtain a more concentrated sample. For maximum yield, 200 µL elutions should be used.

Relative Recovery from 2 Elutions using Different Elution Volumes:

Elution Volume (µL)	50	100	200
% Recovery	85.6	92.3	100.0

Relative Concentration of the First Elution using Different Elution Volumes:

Elution volume (µL)	50	100	200
Relative concentration (%)	100.0	56.7	31.3

5. Storage of DNA

The purified DNA sample may be stored at 4 °C for a few days. It is recommended that samples be placed at –20 °C for long term storage.

Technical Support

Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362. Technical support can also be obtained from our website or through email at techsupport@norgenbiotek.com.