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Blood Genomic DNA Isolation Midi Kit — Product Insert

Product #51400

Norgen's **Blood Genomic DNA Isolation Midi Kit** is designed for the rapid preparation of genomic DNA from 0.3 to 2 mL of whole blood. Purification is based on spin column chromatography as the separation matrix. Norgen's column binds DNA under optimized salt concentrations and releases the bound DNA under low salt and slightly alkali conditions. The purified genomic DNA is fully digestible with all restriction enzymes tested, and is completely compatible with downstream applications including real-time PCR and southern blot analysis.

Norgen's Blood Genomic DNA Isolation Kit allows for the isolation of genomic DNA from the blood of various species, including humans. The genomic DNA is preferentially purified from other cellular proteinaceous components. Typical yields of genomic DNA will vary depending on the cell density of the blood sample. Preparation time for a single sample is less than 45 minutes, and each kit contains sufficient materials for 20 preparations.

Kit Components

Component	Product # 51400 (20 samples)
Lysis Solution	65 mL
Wash Solution I	43 mL
Wash Solution II	2 x 30 mL
Elution Buffer	12 mL
Proteinase K	4.4 mL
Midi Spin Columns	20
Midi Collection Tubes	20
Midi Elution Tubes	20
Product Insert	1

Specifications

Kit Specifications	
Blood Input	0.3-2 mL
Column Binding Capacity	> 100 µg
Average Yield (1 mL of blood)	60 µg*
Time to Complete 10 Purifications	45 minutes

* Yield will vary depending on the type of blood processed

Advantages

- Fast and easy processing using a rapid spin-column format
- Isolate high quality genomic DNA, free from RNA contamination
- Recovered genomic DNA is compatible with various downstream applications

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. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

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.

Customer-Supplied Reagents and Equipment

- Variable speed swing bucket centrifuge that can reach 4500 x g (5000 rpm) and can accommodate 15 mL centrifuge tubes
- Micropipettors
- 96 - 100% ethanol
- 70°C waterbath or incubator

Procedure

All centrifugation steps are carried out in a swing bucket centrifuge that can reach 4500 x g (5000 rpm) and can accommodate 15 mL centrifuge tubes. Various speeds are required for different steps, so please check your centrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\ (r)}}$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

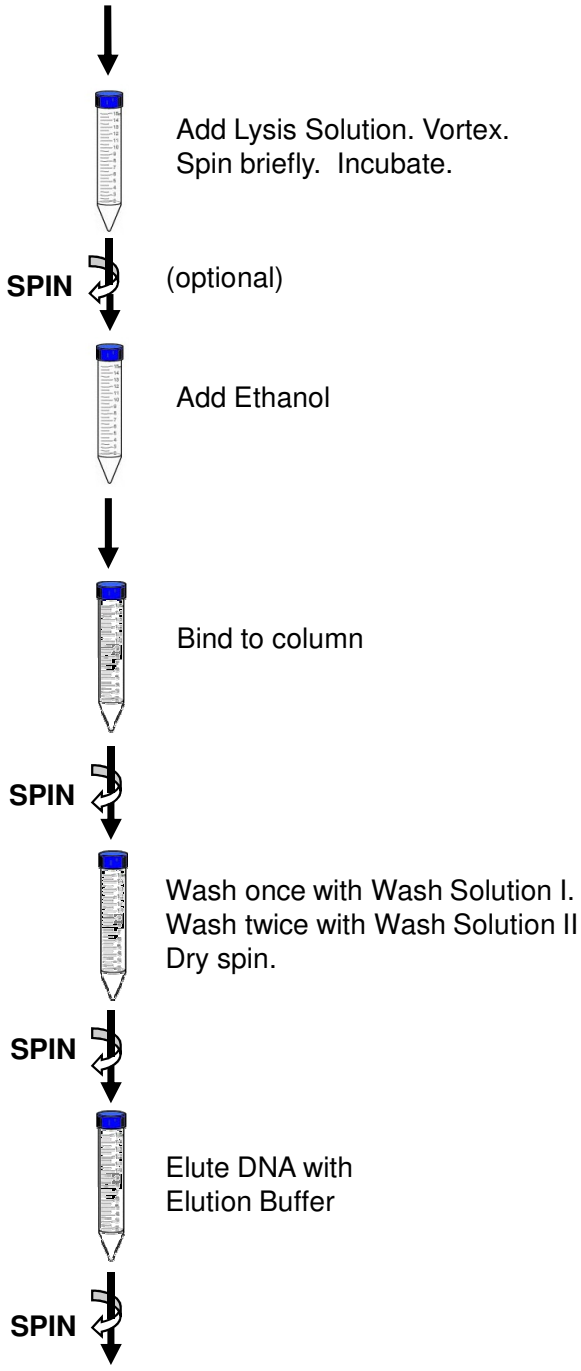
Notes prior to use:

- 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 57 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 100 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Prepare a working concentration of the **Wash Solution II** by adding 70 mL of 96 - 100 % ethanol (provided by the user) to each of the supplied bottles containing the concentrated **Wash Solution II**. This will give a final volume of 100 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- **Always** vortex the Proteinase K before use.

Flow Chart

Procedure for Purifying Blood DNA using Norgen's Blood Genomic DNA Isolation Kit

Obtain anticoagulated blood sample and transfer into a tube containing Proteinase K



Pure Genomic DNA

A. Isolation of DNA from 0.3 - 1 mL of Blood

1. Sample Preparation

a. Add 0.1 mL of **Proteinase K** to a 15 mL tube.
b. Transfer 0.3 - 1 mL of blood sample to the tube containing **Proteinase K**.
c. Add 1.5 mL of **Lysis Solution** to the blood and mix well by inverting 10 times then vortexing for 10 seconds.
d. Briefly spin the tube to collect any drops of liquid from the inside of the lid.
e. Incubate at 70°C for 10 minutes.
f. **(Optional):** 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 g**.
g. Add 1.25 mL of 96-100% Ethanol to the sample and mix well by inversion for 10 times then vortexing for 10 seconds.
h. 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 3 minutes at 1,850 x g (~3,000 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 3 minutes.

3. Column Wash

a. Apply 4.5 mL of **Wash Solution I** (ensure ethanol was added) to the column and centrifuge for 2 minutes at 1,850 x g (~3,000 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 additional 2 minutes.

b. Apply 4.5 mL of **Wash Solution II** (ensure ethanol was added) to the column and centrifuge for 1 minute at 4,500 x g (~5,000 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.

c. Wash column another time by adding 4.5 mL of **Wash Solution II** and centrifuging for 1 minute at 4,500 x g (~5,000 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.

d. Spin the column for 15 minutes in order to thoroughly dry the column at 4,500 x g (~5,000 RPM). Discard the collection tube.

4. DNA Elution

a. Place the column into a provided 15 mL elution tube.
b. Add 0.2 mL of **Elution Buffer** to the column.
c. Incubate at room temperature for 5 minutes.
d. Centrifuge for 2 minutes at 4,500 x g (~5,000 RPM)

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

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.

B. From 1-2 mL of blood

1. Sample Preparation

a. Add 0.2 mL of **Proteinase K** to a 15 mL tube.
b. Transfer 1 - 2 mL of blood sample to the tube containing **Proteinase K**.
c. Add 3 mL of **Lysis Solution** to the blood and mix well by inverting 10 times then vortexing for 10 seconds.
d. Briefly spin the tube to collect any drops of liquid from the inside of the lid.
e. Incubate at 70°C for 10 minutes.
f. **(Optional):** 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 g**.
g. Add 2.5 mL of 96-100% Ethanol to the sample and mix well by inversion for 10 times then vortexing for 10 seconds.
h. 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 half of the lysate to the column and centrifuge for 3 minutes at 1,850 x g (~3,000 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 3 minutes.

d. Repeat **Steps 2b and 2c** to bind the remainder of the lysate.

3. Column Wash

a. Apply 4.5 mL of **Wash Solution I** (ensure ethanol was added) to the column and centrifuge for 2 minutes at 1,850 x g (~3,000 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 additional 2 minutes.

b. Apply 4.5 mL of **Wash Solution II** (ensure ethanol was added) to the column and centrifuge for 1 minute at 4,500 x g (~5,000 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.

c. Wash column another time by adding 4.5 mL of **Wash Solution II** and centrifuging for 1 minute at 4,500 x g (~5,000 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 additional 2 minutes.

d. Spin the column for 15 minutes in order to thoroughly dry the column at 4,500 x g (~5,000 RPM). Discard the collection tube.

4. DNA Elution

a. Place the column into a provided 15 mL elution tube.
b. Add 0.3 mL of **Elution Buffer** to the column.
c. Incubate at room temperature for 5 minutes.
d. Centrifuge for 2 minutes at 4,500 x g (~5,000 RPM)
(Optional): An additional elution may be performed if desired by repeating steps **4a – 4d**. Collect second elution into a new collection tube. The yield can be improved by an additional 20-30% when this second elution is performed.

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.

Related Products	Product #
Blood Genomic DNA Isolation Kit	46300
Blood Genomic DNA Isolation Maxi Kit	31200
Plasma-Serum Circulating DNA Isolation Kit	29900
Plasma-Serum Circulating RNA Isolation Kit	30000
Plasma-Serum Circulating RNA Purification 96-Well Kit (Slurry Format)	29500
Plasma-Serum Circulating Nucleic Acid Purification Kit (Slurry Format)	27800
Leukocyte RNA Purification Kit	21200
HighRanger 1kb DNA Ladder	11900
UltraRanger 1kb DNA Ladder	12100

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 (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
The spin column is clogged.	Inefficient cell lysis	Check **Proteinase K** activity. Also ensure that correct volume of **Lysis Solution** was added to the blood sample.
	Cell debris may be clogging the column	When a high cell number is expected in the blood sample, ensure that the optional spin for 2 minutes at 14,000 rpm after the **Proteinase K** incubation is performed. Take the clean supernatant only for the next binding step.
	The sample is too large	Too many cells were applied to the column. Ensure that **Proteinase K** and **Lysis Solution** are proportionally added as the blood volume is increased. Clogging can be alleviated by centrifuging for a longer period of time until the lysate passes through the column.
The yield of genomic DNA is low	Inefficient cell lysis	Ensure that correct volume of **Lysis Solution** was added to blood sample. Also increase incubation time up to 15 minutes at 70ºC.
	Low DNA binding	Ensure ethanol is added to the sample.
DNA does not perform well in downstream applications.	DNA was not washed three times with the provided Wash Solution	Ensure the column was washed with **Wash Solution I** and **Wash Solution II**.
	Ethanol carryover	Ensure that the dry spin under the Column Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.
	High DNA input used in PCR reaction	For best results, make sure that the final concentration of DNA in the PCR reaction does not exceed 90 ng/uL (1.8 ug DNA per 20 uL PCR reaction)

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