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Blood Genomic DNA Isolation Kit — Product Insert

Product #46300

Norgen's **Blood Genomic DNA Isolation Kit** is designed for the rapid preparation of genomic DNA from up to 500 µL 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 30 minutes, and each kit contains sufficient materials for 50 preparations.

Kit Components

Component	Product # 46300 (50 samples)
Lysis Solution	35 mL
Wash Solution	30 mL
Elution Buffer	12 mL
Proteinase K	1 mL
Spin Columns inserted into Collection Tubes	50
Collection Tubes	50
Elution Tubes	50
Product Insert	1

Specifications

Kit Specifications	
Maximum Blood Input	500 µL
Column Binding Capacity	> 50 µg
Average Yield (200 µL of blood)	3-12 µg*
Time to Complete 10 Purifications	30 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** contains 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

- Benchtop microcentrifuge
- Micropipettors
- 96 - 100% ethanol
- Isopropanol
- 55°C waterbath or incubator

Procedure

All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge 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.

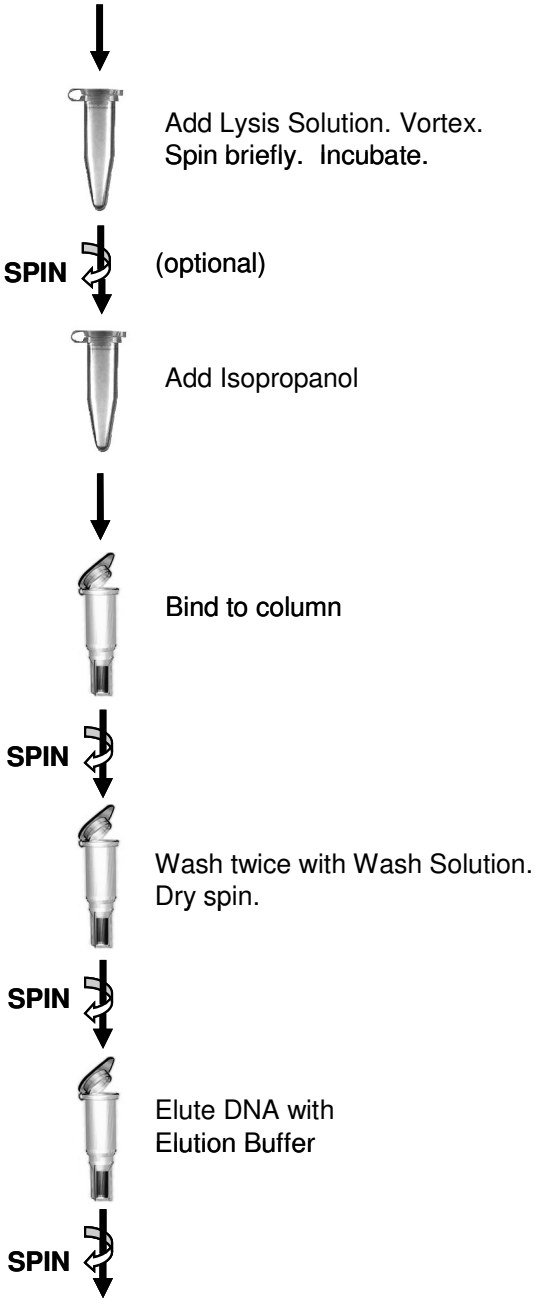
Notes prior to use:

- 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** by adding 70 mL of 95 - 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution**. 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

Procedure

1. Sample Preparation
Note: Please follow either Step A for blood inputs up to 200 µL or Step B for blood inputs of 200 µL to 500 µL.

A. For Blood Inputs up to 200 µL
a. Add 12 µL of **Proteinase K** to a microcentrifuge tube.
b. Transfer up to 200 µL of blood sample to the tube containing **Proteinase K**.
c. Add 600 µL of **Lysis Solution** to the blood and mix well by gentle vortexing for 10 seconds.
d. Briefly spin the tube to collect any drops of liquid from the inside of the lid.
e. Incubate at 55°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. Briefly spin the tube to collect any drops of liquid from the inside of the lid.
h. Add 160 µL of Isopropanol to the sample and mix well by gentle vortexing for 10 seconds.
i. Briefly spin the tube to collect any drops of liquid from the inside of the lid. **Proceed Step 2.**

B. For Blood Inputs from 200 µL to 500 µL
a. Add 12 µL of **Proteinase K** to a microcentrifuge tube.
b. Transfer up to 500µL of blood sample to the tube containing **Proteinase K**.
c. Add 600 µL of **Lysis Solution** to the blood and mix well by gentle vortexing for 10 seconds.
d. Briefly spin the tube to collect any drops of liquid from the inside of the lid.
e. Incubate at 55°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. Briefly spin the tube to collect any drops of liquid from the inside of the lid.
h. Add 240 µL of Isopropanol to the sample and mix well by gentle vortexing for 10 seconds.
i. Briefly spin the tube to collect any drops of liquid from the inside of the lid. **Proceed Step 2.**

2. Sample Binding to Column
a. Obtain a spin column assembled with its collection tube. Apply up to 650 µL of the clarified supernatant to the column and centrifuge for 1 minute at 6,000 x g (~8,000 RPM).
b. 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.

c. Repeat step **2a and 2b** with remained lysate
d. Discard the collection tube containing flow-through.
e. Assemble a spin column with a new collection tube.

3. Column Wash
a. Apply 500 µL of **Wash Solution** (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. Wash column a second time by adding 500 µL of **Wash Solution** and centrifuging for 1 minute at 6,000 x g (~8,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.

c. Spin the column for 2 minutes in order to thoroughly dry the resin 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. 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.

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 Midi Kit	31100
Blood Genomic DNA Isolation Maxi Kit	31200
Plasma-Serum Viral DNA Isolation Kit	29700
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 **Protease 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 55ºC.
	Low DNA binding	Ensure Isopropanol is added to the sample.
DNA does not perform well in downstream applications.	DNA was not washed two times with the provided Wash Solution	Ensure the column was washed with **Wash Solution**.
	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.

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