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Blood DNA Isolation Mini Kit — Product Insert

Product #46300

Norgen's **Blood DNA Isolation Mini Kit** is designed for the rapid preparation of genomic DNA from up to 200 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 PCR, real-time PCR, Long-Range PCR, RFLP analysis used for paternity testing and southern blot analysis.

Norgen's Blood DNA Isolation Mini 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 or 100 preparations.

Kit Components

Component	Product # 46300 (50 samples)
Lysis Buffer B	20 mL
Solution WN	18 mL
Wash Solution A	18 mL
Elution Buffer B	30 mL
Proteinase K in Storage Buffer	1.2 mL
Spin Columns	50
Collection Tubes	50
Elution Tubes	50
Product Insert	1

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

Specifications

Kit Specifications	
Minimum Blood Input	20 µL
Maximum Blood Input	200 µL
Column Binding Capacity	> 50 µg
Average Yield (200 µL of blood)	4-12 µg*
Time to Complete 10 Purifications	30 minutes

* Yield will vary depending on the type of blood processed

Storage Conditions and Product Stability
All solutions should be kept tightly sealed and stored at room temperature. This kit is stable for 2 years after the date of shipment. The kit contains a ready-to-use Proteinase K, which is dissolved in a specially prepared storage buffer.

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*.

Lysis Buffer B and **Solution WN** 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.

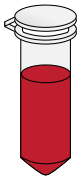
Customer-Supplied Reagents and Equipment
- Benchtop microcentrifuge
- Micropipettors
- 2 mL microcentrifuge tubes
- 96 - 100% ethanol
- 55°C water bath or incubator
- Vortex
- RNase A (if RNA-free DNA is required)
- Lysozyme (for blood containing Gram positive bacterial pathogens)
- 37°C incubator (for blood containing Gram positive bacterial pathogens)
- PBS (optional)

Flow Chart

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

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

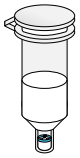
BIND



Add Lysis Buffer B.
Vortex. Incubate.

SPIN

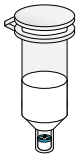
Add ethanol. Vortex.



Bind to Column

WASH

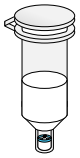
SPIN



Wash once with Wash Solution WN.
Wash twice with Wash Solution A.
Dry spin.

ELUTE

SPIN



Elute DNA with
Elution Buffer B

SPIN

Pure Genomic DNA

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 **Solution WN** by adding 24 mL of 96 - 100 % ethanol (provided by the user) to each supplied bottle containing the concentrated **Solution WN**. This will give a final volume of 42 mL. The labels on the bottles have a box that may be checked to indicate that the ethanol has been added.
- Prepare a working concentration of the **Wash Solution A** by adding 42 mL of 96 - 100 % ethanol (provided by the user) to each supplied bottle containing the concentrated **Wash Solution A**. This will give a final volume of 60 mL. The labels on the bottles have a box that may be checked to indicate that the ethanol has been added.
- **For blood containing Gram positive bacterial pathogens**, prepare a 400 mg/mL stock solution (approximately 1.7 x 10^7 units/mL) of lysozyme as per supplier's instructions.
- **Always** vortex the Proteinase K before use.
- RNase A (not provided) can be used if RNA-free DNA is required. Use RNase A at the indicated optional step in the protocol.
- This kit can be used to process up to 400 µL blood. It is important to increase the volumes of the reagents during the sample preparation step proportionally (Proteinase K, Lysis Buffer B and Ethanol), and then follow the typical procedures for column binding, wash and elution. For example, to process 400 µL of blood, double the amounts written below for the Proteinase K, Lysis Buffer B and Ethanol.

1. Sample Preparation

NOTE: For DNA isolation from blood containing Gram positive bacterial pathogens, please see Appendix A for Sample Preparation.

a. Add 20 µL of **Proteinase K** (vortex before use) to a microcentrifuge tube.

b. Transfer 20 - 200 µL of blood sample to the tube containing **Proteinase K**.

Note: When using volumes less than 200 µL, add a suitable volume of PBS to bring the volume up to 200 µL.

Optional RNase A Treatment:
If RNA-free genomic DNA is required, add the equivalent of 100 units of RNase A (not to exceed 10 μL) to the lysate.

c. Add 300 μL of Lysis Buffer B to the blood and mix well by vortexing for 10 seconds.
d. 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**.

e. Briefly spin the tube to collect any drops of liquid from the inside of the lid.
f. Add 110 μL of 96-100% Ethanol to the sample and mix well by vortexing for 10 seconds.
g. 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 **Solution WN** (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 A** (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 A** 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 B** 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 and Concentration using Different Elution Volumes:

Elution Volume (μL)	50	100	200
Recovery (%) from 2 elutions	85.6	92.3	100.0
Concentration (%) of the first elution	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.

Appendix A

Notes prior to use:

- Prepare a 400 mg/mL stock solution (approximately 1.7 x 10^7 units/mL) of lysozyme as per supplier's instructions.

Sample Preparation for Blood Containing Gram Positive Bacterial Pathogens

a. Add 20 μL of **Lysozyme** to a microcentrifuge tube, and transfer 20 μL - 200 μL of blood sample to the tube containing **Lysozyme**

b. Mix well by vortexing, and incubate at 37°C for 1 hour. (Note: 0.5 to 2 hours incubation time can be used depending on the bacterial strain being lysed).

c. After incubation, add 20 μL of **Proteinase K** (vortex before use) to the tube and proceed to **Step 1c**.

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 Buffer B** 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 Buffer B** 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 Buffer B** was added to blood sample. Also increase incubation time up to 15 minutes at 55ºC. Ensure that the correct amount of 96-100% ethanol was added to the lysate before binding to the column.
DNA does not perform well in downstream applications.	DNA was not washed with the provided Solution WN and Wash Solution A	Ensure the column was washed once with **Solution WN** and twice with **Wash Solution A**.
	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.

Related Products	Product #
Blood DNA Isolation Midi Kit	51400
Blood DNA Isolation Maxi Kit	31200
Blood DNA Isolation 96-Well Kit	46350
Dried Blood Spot Genomic DNA Isolation Kit	36000
RNase A	26260

Technical Support
Contact our Technical Support Team between the hours of 9:00 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 support@norgenbiotek.com.

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