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Blood DNA Isolation 96-Well Kit — Product Insert

Product # 46350

Norgen's **Blood DNA Isolation 96-Well Kit** provides a rapid method for the high-throughput isolation of genomic DNA from up to 200 µL of whole blood. Purification is based on 96-well column chromatography as the separation matrix. Norgen's 96-well plate 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 96-Well 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 96-well plate is less than 90 minutes, and each kit contains sufficient materials for 192 preparations.

Kit Components

Component	Product #46350 (192 preps)
Lysis Buffer B	2 x 40 mL
Solution WN	55 mL
Wash Solution A	2 x 38 mL
Elution Buffer B	2 x 30 mL
Proteinase K in Storage Buffer	4.4 mL
96-Well Plate	2
Adhesive Tape	4
96-Well Collection Plate	2
96-Well Elution Plate	2
Product Insert	1

Specifications

Kit Specifications	
Maximum Blood Input	200 µL
Average Yield (200 µL of blood)	2-8 µg*
Time to Complete 96 Purifications	45 minutes

* Yield will vary depending on the type of blood processed

Advantages

- Fast and easy processing using either a vacuum manifold or centrifugation
- Isolate high quality genomic DNA
- Isolate genomic DNA from various species, including humans
- Recovered genomic DNA without RNA contamination is compatible with various downstream applications

Storage Conditions and Product Stability

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

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 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

You must have the following in order to use the Blood Genomic DNA Isolation 96-Well Kit:

- 1.5 mL microcentrifuge tubes
- 96 - 100% ethanol
- Benchtop microcentrifuge
- Collection/Waste Tray for vacuum manifold. Two 96-Well Collection Plates are provided with the kit.
- 56°C waterbath or incubator
- Vortex
- Nuclease-free water (for volume adjustment of samples < 200 μL)
- Lysozyme (for blood containing Gram positive bacterial pathogens)
- 37°C incubator (for blood containing Gram positive bacterial pathogens)
- For ***Vacuum Format***:
    - Vacuum manifold with vacuum pump capable of generating a minimum pressure of -650 mbar or -25 in. Hg (such as Whatman UniVac 3 Vacuum to Collect Manifold)
    - Sealing tape or pads
- For ***Centrifuge Format***:
    - Centrifuge with rotor for 96-well plate assembly, such as AllSpin Js-5.3 Rotor for Avanti® J-26xp centrifuge, Beckman Coulter or similar rotor that can hold the stack of the 96-well Plate and the 96-Well Collection Plate and that can reach the minimum speed of 4000 rpm (~4000xg)

Flow Chart

Procedure for Purifying Blood DNA using Norgen's Blood Genomic DNA Isolation 96-Well Kit

Add Proteinase K and blood sample to a microcentrifuge tube

↓

Add Lysis Buffer B, mix.
Incubate for 10 minutes at
55°C. Add ethanol. Mix

↓

Bind

↓

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

↓

Elute DNA with
Elution Buffer B

↓

Pure Blood Genomic DNA

Procedure

For *Vacuum Manifold*: All vacuum steps are performed at room temperature. The correct pressure can be calculated using the conversions:

1 **mbar** = 100 **Pa** = 0.0394 **in. Hg** = 0.75 **mm Hg** = 0.0145 **psi**

For *Centrifugation*: 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.
- The isolation of DNA from whole blood, plasma and serum can be performed using either a vacuum manifold or centrifugation. For purification using vacuum, please follow the procedure outlined in Section 2A. For purification using centrifugation, please follow the procedure outlined in Section 2B. Lysate preparation is the same for both procedures.
- 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.
- The volumes stated in each procedure for lysate preparation are the volumes required to prepare samples for each well of the 96-well plate.
- Prepare a working concentration of **Solution WN** by adding 73 mL of **96-100% ethanol** (provided by user) to the supplied bottle containing concentrated **Solution WN**. This will give a final volume of 128 mL. The label on the bottle has a box that can be checked to indicate that ethanol has been added.
- Prepare a working concentration of **Wash Solution A** by adding 90 mL of 96 - 100% ethanol (provided by the user) to the supplied bottles containing concentrated **Wash Solution A**. This will give a final volume of 128 mL. The label on the bottle has a box that can be checked to indicate that 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.

1. Lysate Preparation

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

a. Add 20 µL of **Proteinase K** (vortex before use) to a microcentrifuge tube.
b. Transfer up to 200 µL of blood or plasma sample to the tube containing **Proteinase K**.
c. If the blood input volume is less than 200 µL, add Nuclease-free water to bring the blood volume up to 200 µL.

d. Add 300 µL of **Lysis Buffer B** to the sample and mix well by gentle vortexing for 10 seconds.
e. Briefly spin the tube to collect any drops of liquid from the inside of the lid.
f. Incubate at 56°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 g**.

g. Add 250 µL of 96-100% ethanol and mix by gentle vortexing.
h. Briefly spin the tube to collect any drops of liquid from the inside of the lid.

2. Genomic DNA Isolation

Note: The purification of genomic DNA from the lysate prepared in Section 1 could be performed using either a vacuum manifold or centrifugation. For purification using vacuum, please follow the procedure outlined in 2A. For purification using centrifugation, please follow the procedure outlined in 2B

A. Genomic DNA isolation Using Vacuum Manifold

1. Binding DNA to 96-Well Plate

a. Assemble the 96-Well Plate and the vacuum manifold according to manufacturer's recommendations.
b. Apply the lysate into each well of the 96-Well Plate. Tape the plate or any unused wells using sealing tape or pads (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for 2 minutes.

Note: Depending on the starting material, a small quantity of precipitates may appear in the lysate-ethanol mix. No additional step is required to remove these precipitates prior to application of the mixture to the wells.

c. Turn off vacuum and ventilate the manifold. Discard the flowthrough. Reassemble the 96-Well Plate and the vacuum manifold.

Note: Ensure that all of the lysate from each well has passed through into the collection/waste tray. If the entire lysate volume has not passed, apply vacuum for an additional 2 minutes.

2. DNA Wash

a. Apply 500 µL of **Solution WN** to each well of the 96-Well Plate. Tape the plate or any unused wells using sealing tape or pads (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for 2 minutes.

Note: Ensure the entire wash solution has passed through into the collection/waste tray by inspecting the 96-Well Plate. If the entire wash volume has not passed, apply vacuum for an additional 2 minutes.

b. Turn off vacuum and ventilate the manifold. Discard the flowthrough.
c. Reassemble the 96-Well Plate and the vacuum manifold.

d. Apply 500 μL of **Wash Solution A** to each well of the 96-Well Plate. Tape the plate or any unused wells and apply vacuum for 2 minutes.
e. Turn off vacuum and ventilate the manifold. Discard the flowthrough Pat the bottom of the 96-Well Plate gently to remove any residual wash buffer. Reassemble the 96-Well Plate and the vacuum manifold.
f. Repeat steps **3d** and **3e** to wash the plate for a third time.
g. Apply vacuum for an additional 5 minutes in order to completely dry the plate.
h. Turn off vacuum and ventilate the manifold.

3. DNA Elution

a. Replace the collection/waste tray in the vacuum manifold with the provided 96-Well Elution Plate. Complete the vacuum manifold assembly with the 96-Well Plate.
b. Add 200 μL of **Elution Buffer B** to each well of the plate.
c. Apply vacuum for 3 minutes.

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.

4. Storage of DNA

Use the provided adhesive tape to seal the 96-Well Elution Plate. The purified DNA samples may be stored at –20°C for a few days. It is recommended that samples be placed at –70°C for long term storage.

B. Genomic DNA Purification Using Centrifugation

Note: To purify genomic DNA using a vacuum manifold please follow Section A above.

1. Binding DNA to 96-Well Plate

a. Place the 96-Well Plate on top of the 96-Well Collection Plate.

Note: The user should ensure that the assembled 96-Well Plate and the 96-Well Collection Plate stack fits into the rotor without interfering with the centrifugation process.

b. Apply the lysate into each well of the 96-Well Plate. Centrifuge the assembly at 5,000 RPM (or a minimum of 4,000 RPM) for 4 minutes.
c. Discard the flowthrough. Reassemble the 96-Well Plate and the 96-Well Collection Plate.

Note: Ensure that all of the lysate from each well has passed through into the 96-Well Collection Plate. If the entire lysate volume has not passed, centrifuge for an additional 2 minutes.

2. DNA Wash

a. Apply 500 μL of **Solution WN** to each well of the 96-Well Plate. Centrifuge the assembly at 5,000 RPM (or a minimum of 4,000 RPM) for 2 minutes.

Note: Ensure the entire wash solution has passed through into the 96-Well Collection Plate by inspecting the 96-Well Plate. If the entire wash volume has not passed, centrifuge for an additional 2 minutes.

b. Discard the flowthrough. Reassemble the 96-Well Plate and the 96-Well Collection Plate.
c. Apply 500 µL of **Wash Solution A** to each well of the 96-Well Plate.
d. Centrifuge the assembly at 5,000 RPM (or a minimum of 4,000 RPM) for 2 minutes.
e. Discard the flowthrough. Reassemble the 96-Well Plate and the 96-Well Collection Plate.
f. Repeat steps **3c** and **3d** to wash the 96-Well plate for a third time.
g. Discard the flowthrough. Pat the bottom of the 96-Well Plate dry. Reassemble the 96-Well Plate and the 96-Well Collection Plate. Centrifuge the assembly at 5,000 RPM (or a minimum of 4,000 RPM) for 15 minutes in order to completely dry the 96-Well plate.

3. DNA Elution

a. Stack the 96-Well Plate on top of the 96-Well Elution Plate.
b. Add 200 µL of **Elution Buffer B** to each well of the 96-Well Plate.
c. Centrifuge the assembly at 5,000 RPM (or a minimum of 4,000 RPM) for 3 minutes.

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.

4. Storage of DNA

Use the provided adhesive tape to seal the 96-Well Elution Plate. The purified DNA samples may be stored at –20°C for a few days. It is recommended that samples be placed at –70°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 up to 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 of Lysate Preparation**.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
The 96 well Plate is clogged.	Cell debris may be clogging the 96-Well Plate	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.
	The sample is too large	Too many cells were applied to the well. 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 well.
	Insufficient Vacuum	Ensure that a vacuum pressure of at least -650 mbar or -25 in. Hg is developed
	Centrifuge temperature too low	Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 15°C may cause precipitates to form that can cause the wells to clog.
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 56ºC.
	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 Solution WN and Wash Solution A	Ensure the 96-Well Plate was washed one time with **Solution WN** and two times with **Wash Solution A**. An additional wash with **Wash Solution A** can improve DNA performance in downstream applications, however it may reduce DNA yield.
	Ethanol carryover	Ensure that the dry spin under the 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 Micro Kit	52100
Blood DNA Isolation Mini Kit	46300
Blood DNA Isolation Midi Kit	51400
Blood DNA Isolation Maxi Kit	31200
Dried Blood Spot Genomic DNA Isolation Kit	36000
HighRanger 1kb DNA Ladder	11900
UltraRanger 1kb DNA Ladder	12100

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 techsupport@norgenbiotek.com.

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