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Blood Genomic DNA Isolation Mini Kit Dx

Product Insert

REF **Dx46300** CE IVD PIDx46300-2

Intended Use

Norgen's Blood Genomic DNA Isolation Mini Kit Dx is designed for the rapid preparation of genomic DNA from up to 200 µL of whole blood for subsequent *in vitro* diagnostic use. Both fresh and frozen anticoagulated blood may be used with this procedure. 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.

This kit is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of DNA followed by signal detection or amplification. Any diagnostic results generated using the DNA isolated with Norgen's Blood Genomic DNA Isolation Mini Kit Dx in conjunction with an *in vitro* diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, suitable controls for downstream applications should be used.

Norgen's Blood Genomic DNA Isolation Mini Kit Dx is intended for use by professional users such as technicians, physicians and biologists experienced and trained in molecular biological techniques including experience with whole blood samples and DNA isolation.

Norgen's Blood Genomic DNA Isolation Mini Kit Dx does not provide a diagnostic result. It is the sole responsibility of the user to use and validate the kit in conjunction with a downstream *in vitro* diagnostic assay.

Kit Components

Component	Product # Dx46300 (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

Label Legend

								
Do not reuse	Use by	Batch Code	Catalogue Number	Contains sufficient for <n> tests	Manu-facturer	In Vitro Diagnostic Medical Device	Consult instructions for use	Temper-ature limitation

Made in Canada
Norgen Biotek

Advantages

- CE-IVD marked in accordance with EU Directive 98/79/EC
- Fits into *in vitro* diagnostic workflows
- 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. All solutions and plastics can be used until the expiration date specified on their labels.

Precautions

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

- Benchtop microcentrifuge
- Micropipettors
- 2 mL microcentrifuge tubes
- 96 - 100% ethanol
- 55°C waterbath or incubator

Procedure

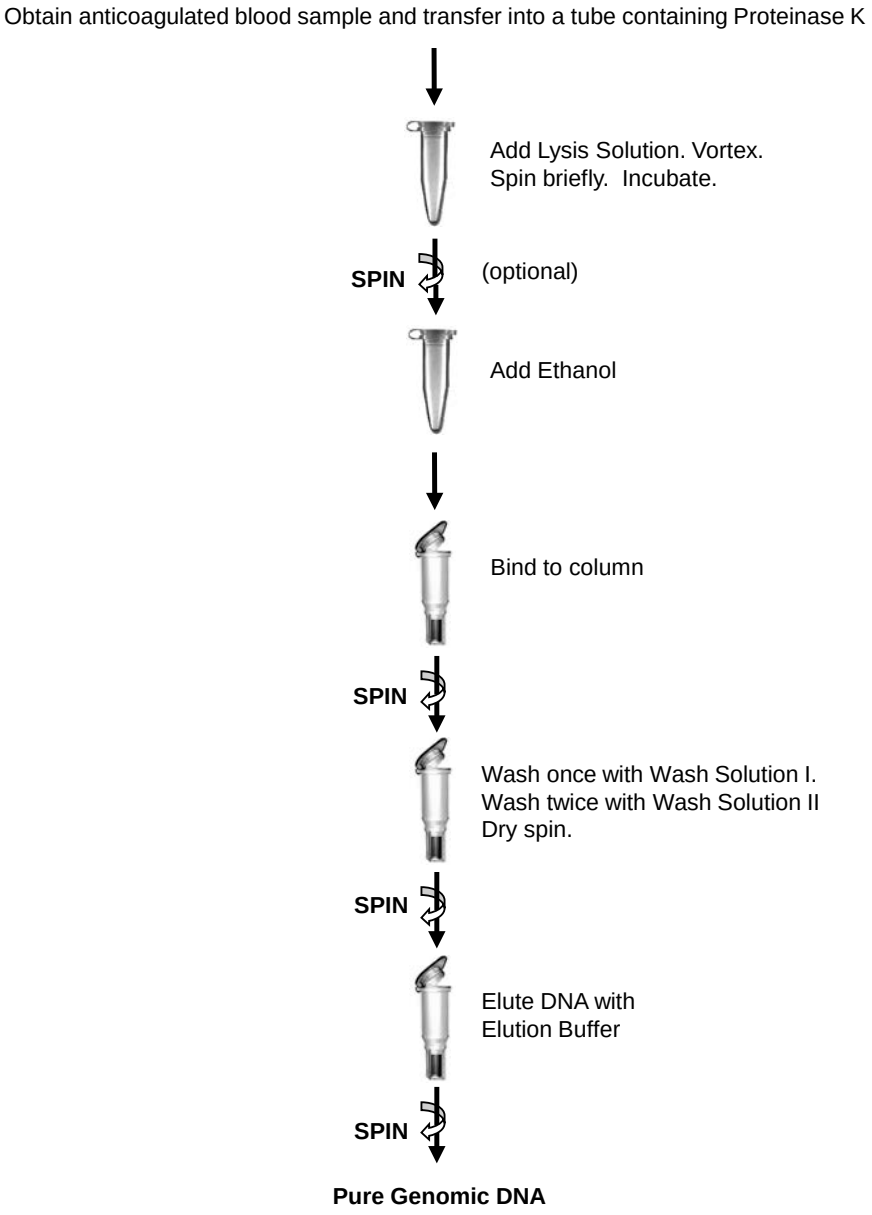
All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can 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.

Flow Chart

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



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. 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 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. 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.

1. Sample Preparation

a. Add 20 µL of **Proteinase K** to a microcentrifuge tube.
b. Transfer 20 - 200 µL of blood sample to the tube containing **Proteinase K**.
c. Add 300 µL of **Lysis Solution** to the blood and mix well by 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 250 µL of 96-100% Ethanol to the sample and mix well by vortexing for 10 seconds.
i. 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.

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 Ethanol is added to the sample.
DNA does not perform well in downstream applications.	DNA was not washed with the provided Wash Solution	Ensure the column was washed once with **Wash Solution I** and **twice 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.

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.

Product Use Restriction

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The respective user is liable for any and all damages resulting from application of Norgen's Blood Genomic DNA Isolation Mini Kit Dx for use deviating from the intended use as specified in the user manual.

All products sold by Norgen Biotek are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately. The kit contents are for laboratory use only, and they must be stored in the laboratory and must not be used for purposes other than intended. The kit contents are unfit for consumption.

Authorized Representative

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