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Dried Blood Spot DNA Isolation Kit — Product Insert

Product #36000

Norgen's **Dried Blood Spot DNA Isolation Kit** is designed for the rapid preparation of total genomic DNA from dried blood spots. Purification is based on spin column chromatography. Norgen's column binds DNA under high salt concentrations and releases the bound DNA under low salt and slightly alkaline conditions. The genomic DNA is preferentially purified from RNA and cellular proteinaceous components. The purified genomic DNA is fully digestible with all restriction enzymes tested, and is completely compatible with downstream applications including Southern Blot analysis.

The **Dried Blood Spot DNA Isolation Kit** allows for the isolation of genomic DNA from the blood of various species, including humans. The blood should be spotted and dried on suitable filter paper or specimen collection cards. Typical yields of genomic DNA will vary depending on the cell density of the blood sample. Preparation time for a single sample is 30 minutes, and each kit contains sufficient materials for 50 preparations.

Kit Components

Component	Product # 36000 (50 samples)
Digestion Buffer B	6 mL
Lysis Buffer B	20 mL
Wash Solution WN	18 mL
Wash Solution A	18 mL
Elution Buffer B	15 mL
Proteinase K	1.2 mL
Micro Spin Columns	50
Collection Tubes	50
Elution Tubes	50
Product Insert	1

Specifications

Kit Specifications	
Blood Spots Input	3 x 3 mm (1/8 inch) diameter punches
Column Binding Capacity	> 50 µg
Average Yield	150 ng*
Time to Complete 10 Purifications	35 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
- Isolate genomic DNA from anticoagulated and untreated blood
- 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. This kit is stable for 1 year after the date of shipment. The kit contains a ready-to-use Proteinase K, which is dissolved in a specially prepared storage buffer. The buffered Proteinase K is stable for up to 1 year after the date of shipment when stored at room temperature.

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 **Digestion Buffer B**, **Lysis Buffer B** and **Wash 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
- 3 mm single-hole paper puncher
- Benchtop microcentrifuge
- Micropipettors
- 1.5 mL microcentrifuge tubes
- 96 - 100% ethanol
- 56°C waterbath or incubator
- 85°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 Dried Blood Spot DNA Isolation Kit

Place 3 punched-out circles from a dried blood spot into a 1.5 mL microcentrifuge tube. Add Digestion Buffer B. Vortex. Incubate at 85°C for 10 minutes

Add Proteinase K and Lysis Buffer B. Vortex. Incubate at 56°C for 10 minutes.

SPIN (optional)

Add Ethanol

Bind to column

SPIN

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

SPIN

Elute DNA with Elution Buffer B

SPIN

Pure Genomic DNA

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.
- Prepare a working concentration of the **Wash Solution WN** by adding 24 mL of 96 - 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution WN**. 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 A** by adding 42 mL of 96 - 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution A**. 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. Cut out 3 x 3 mm (1/8 inch) diameter circles from a dried blood spot with a single-hole paper puncher and place in a clean microcentrifuge tube.

Note: Up to 6 x 3 mm diameter circles can be used to obtain higher DNA yield.

b. Add 100 µL of the **Digestion Buffer B** and vortex for 10 seconds.
c. Incubate at 85°C for 10 minutes.
d. Briefly spin the tube to collect any drops of liquid from the inside of the lid.
e. Add 20 µL of **Proteinase K** to a microcentrifuge tube.
f. Add 300 µL of **Lysis Buffer B** to the blood 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.
h. Incubate at 56°C for 10 minutes.
i. Briefly spin the tube to collect any drops of liquid from the inside of the lid.
j. Add 250 µL of 96-100% ethanol to the sample and mix well by vortexing for 10 seconds.
k. 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 clear lysate to the column and centrifuge for 1 minute at 6,000 x g (~8,000 RPM).

Note: Do not transfer the remaining filter paper or collection card debris to the column, transfer only the clear lysate.

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 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 20 µL – 100 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).

e. Perform a second elution by transferring the column into a clean 1.7 mL tube and repeating steps 4-a through 4-d.

Table 1: Relationship between Elution Volume, Expected Yield and Concentration

Elution volume (µL)	20	30	50	100
Expected total yield from 2 elutions (%)	41.5	47.8	51.7	100.0
Expected Average concentration from the 2 elutions (%)	100.0	76.7	49.8	48.2

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 Midi Kit	51400
Blood Genomic DNA Isolation Maxi Kit	31200
Plasma/Serum Circulating DNA Purification Mini Kit	50600
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 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 digestion	Ensure that the correct volume of **Digestion Buffer B** was added to the sample followed by incubation for 10 minutes at 85ºC.
	Inefficient cell lysis	Ensure that correct volume of **Lysis Buffer B** was added to the 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 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.

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