

Blood Genomic DNA Isolation Kit

Product # 18200

Product Insert

Norgen's **Blood Genomic DNA Isolation Kit** is designed for the rapid preparation of genomic DNA from up to 200 μ L of whole blood, plasma and serum. Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. Norgen's resin binds DNA under high 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 Southern Blot analysis.

The 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 # 18200 (50 samples) |
|--------------------|------------------------------|
| Lysis Solution | 20 mL |
| Binding Solution | 6 mL |
| Wash Solution I | 30 mL |
| Wash Solution II | 39 mL |
| Elution Buffer | 12 mL |
| Proteinase K | 13 mg |
| Micro Spin Columns | 50 |
| Collection Tubes | 100 |
| Product Insert | 1 |

Specifications

| Kit Specifications | |
|--------------------------------------|--------------|
| Maximum Blood Input | 200 μ L |
| Average Yield (200 μ L of blood) | 3-5 μ g* |
| 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
- Isolate genomic DNA from various inputs including plasma, whole blood and serum
- 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.

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 **Binding 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.

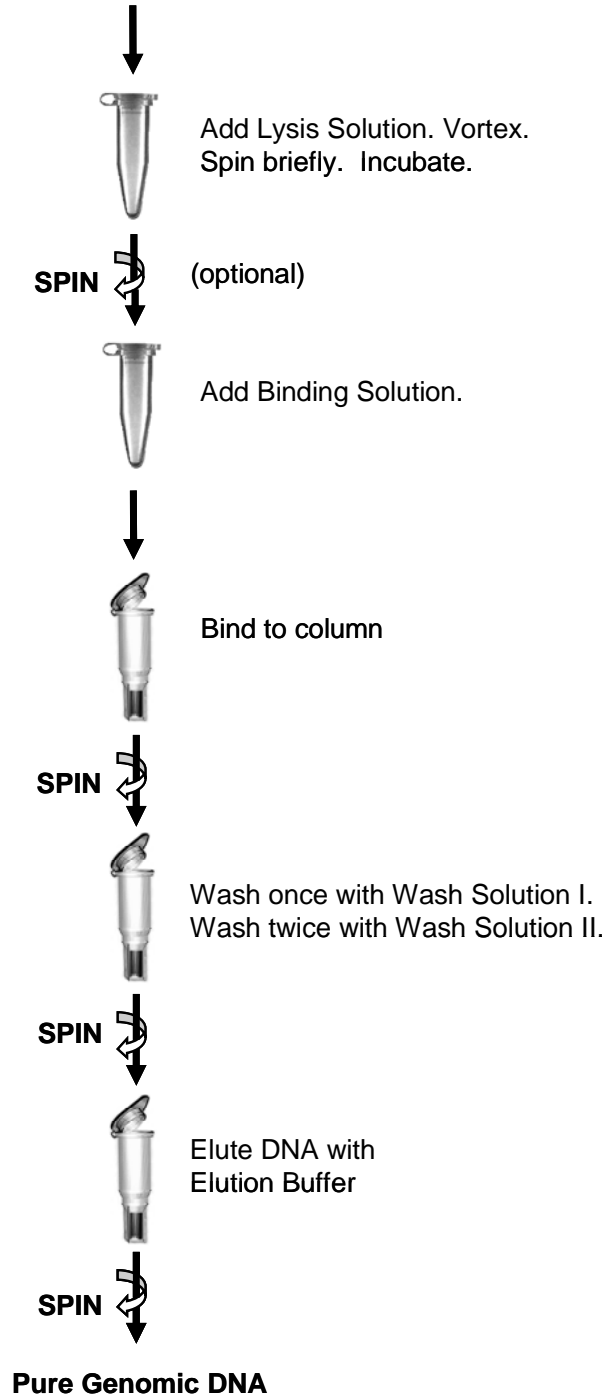
Customer-Supplied Reagents and Equipment

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

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



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.
- Reconstitute the Proteinase K in 0.65 mL of molecular biology grade water, aliquot in 120 μ L fractions, and store the unused portions at -20°C until needed.
- Prepare a working concentration of **Binding Solution** by adding 3.5 mL of Isopropanol (provided by user) to the supplied bottle containing concentrated **Binding Solution**. This will give a final volume of 9.5 mL. The label on the bottle has a box that can be checked to indicate that Isopropanol has been added.
- Prepare a working concentration of **Wash Solution II** by adding 21 mL of 96 - 100% ethanol (provided by the user) to the supplied bottle containing concentrated **Wash Solution II**. This will give a final volume of 60 mL. The label on the bottle has a box that can be checked to indicate that ethanol has been added.

1. Sample Preparation

- a. Add 12 μ L of **Proteinase K** to a microcentrifuge tube.
- b. Transfer up to 200 μ L of blood sample to the tube containing **Proteinase K**.
- c. Add 350 μ L 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. Add 150 μ L of **Binding Solution** (ensure Isopropanol was added) and mix by gentle vortexing.
- h. Briefly spin the tube to collect any drops of liquid from the inside of the lid.

2. Sample Binding to Column

- a. Assemble a spin column with a provided collection tube. Apply up to 650 μL of the clarified supernatant to the column and centrifuge for 2 minutes at 14,000 x g (~14,000 RPM).

Note: Ensure the entire sample has passed through into the collection tube by inspecting the column. If the entire sample volume has not passed, spin for additional 2 minutes.

- b. Discard the collection tube containing flow-through.
- c. Assemble a spin column with a provided new collection tube.

3. Column Wash

- a. Apply 500 μL of **Wash Solution I** 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.

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 II** (ensure ethanol was added) 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.
- c. Wash column a third time by adding another 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 resin. Discard the collection tube.

4. DNA Elution

- a. Place the column into a fresh 1.7 mL DNase-free microcentrifuge tube (provided by the user).
- b. Add 200 μL of **Elution Buffer** to the column.
- c. Centrifuge for 2 minutes at **200 x g (~2,000 RPM)**, followed by a 2 minute spin at **14,000 x g (~14,000 RPM)**.
- d. **(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.

Troubleshooting Guide

| Problem | Possible Cause | Solution and Explanation |
|---|---|--|
| The micro spin column is clogged. | Inefficient cell lysis | Check Protease K activity. Be sure to store the stock solution at -20°C immediately after use. 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 55°C. |
| | Low DNA binding | Ensure Isopropanol is added to the Binding Solution . |
| DNA does not perform well in downstream applications. | DNA was not washed three times with the provided Wash Solutions | Ensure the column was washed one time with Wash Solution I and two times with Wash Solution II . An additional wash with Wash Solution II can improve DNA performance in downstream applications, however it may reduce DNA yield. |
| | 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 # |
|----------------------------------|------------------|
| HighRanger 1kb DNA Ladder | 11900 |
| UltraRanger 1kb DNA Ladder | 12100 |
| Milk Bacterial DNA Isolation Kit | 21500 |
| Saliva DNA Isolation Kit | 21410 |
| Urine Bacteria DNA Isolation Kit | 21700 |

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.

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