Norgen’s Blood Genomic DNA Isolation Kit (Magnetic Bead System) is designed for the rapid preparation of genomic DNA from up to 200 µL of whole blood from various species, including human. Purification is based on Magnetic bead as the separation matrix. Norgen’s Magnetic beads bind DNA under optimized salt concentrations and releases the bound DNA under low salt and slightly alkali conditions. 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. The purified genomic DNA is fully digestible with all restriction enzymes tested, and is completely compatible with downstream applications including real-time PCR, NGS and microarray analysis.

**Norgen’s Purification Technology**

Purification is based on the use of magnetic beads that bind DNA under optimized binding conditions. Norgen’s Blood DNA Isolation Kit (Magnetic Bead System) allows for the isolation of genomic DNA from various different blood samples, including human. Lysis Buffer B and Proteinase K are added to the sample, mixed and incubated at 55°C to lyse the cells. Magnetic Bead Suspension and ethanol are then added to the clean supernatant, and the resulting solution is placed on the magnetic separation rack. Only the DNA will bind to the magnetic beads, while most of the proteins will be removed in the supernatant. The bound DNA is then washed with Solution WN and 70% ethanol in order to remove any remaining impurities, and the purified total DNA is eluted with the Elution Buffer B. The purified DNA can be used in a number of downstream applications.

### Specifications

<table>
<thead>
<tr>
<th>Kit Specifications</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Preps</td>
<td>50</td>
</tr>
<tr>
<td>Maximum Blood Input</td>
<td>200 µL</td>
</tr>
<tr>
<td>Average Yield (200 µL of blood)*</td>
<td>4-12 µg</td>
</tr>
<tr>
<td>Average Purity (OD260/280)</td>
<td>1.7-1.9</td>
</tr>
<tr>
<td>Time to Complete 12 Purifications</td>
<td>40 minutes (hands-on time)</td>
</tr>
</tbody>
</table>

* Average DNA yield will vary depending on the type of samples

### Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature (15 – 25°C). These reagents should remain stable for at least 2 years in their unopened containers. The kit contains a ready-to-use Proteinase K solution, which is dissolved in a specially prepared storage buffer. The Proteinase K is stable for up to 2 years after delivery when stored at room temperature. To prolong the lifetime of Proteinase K, storage at 2–8°C is recommended.

### Advantages

- Isolate genomic DNA from cultured cells as well as various tissue types
- Fast, reproducible and easy processing using a Magnetic bead system
- Isolate high quality genomic DNA
- Recovered genomic DNA is compatible with various downstream applications
Kit Components

<table>
<thead>
<tr>
<th>Component</th>
<th>Product # 59800 (50 samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysis Buffer B</td>
<td>20 mL</td>
</tr>
<tr>
<td>Proteinase K</td>
<td>1.2 mL</td>
</tr>
<tr>
<td>Magnetic Bead Suspension</td>
<td>2 x 1.1 mL</td>
</tr>
<tr>
<td>Solution WN</td>
<td>18 mL</td>
</tr>
<tr>
<td>Elution Buffer B</td>
<td>15 mL</td>
</tr>
<tr>
<td>Elution tubes (1.7 mL)</td>
<td>50</td>
</tr>
<tr>
<td>Product Insert</td>
<td>1</td>
</tr>
</tbody>
</table>

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 2 year in their unopened containers. The kit contains a ready-to-use Proteinase K solution, which is dissolved in a specially prepared storage buffer. The Proteinase K is stable for up to 2 year after delivery when stored at room temperature. To prolong the lifetime of Proteinase K, storage at 2–8°C is recommended.

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 contains guanidinium salts, and should be handled with care. Guanidinium salts forms 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
- Magnetic bead separation rack
- Micropipettors
- Microcentrifuge tube
- 70% ethanol (prepare fresh)
- 96-100% ethanol
- Temperature adjustable (37°C, 55°C, 65°C) incubator(s)
- Nuclease-free water
- Lysozyme (400 mg/mL) (For blood containing Gram positive bacterial pathogens)
Flow Chart
Procedure for Purifying Blood DNA using Norgen’s Blood DNA Isolation Kit (Magnetic Bead System)

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

Add Lysis Buffer B. Mix and incubate at 55°C for 10 minutes.

Add Ethanol and Magnetic Bead Suspension. Mix and incubate for 10 minutes.

Place tube in magnetic separation rack. Let stand for 1 minute.

Discard supernatant. Add Solution WN, mix and incubate for 1 minute.

Discard supernatant. Add 70% ethanol, mix and incubate for 1 minute.

Repeat ethanol wash step. Incubate open tube at 65°C for 5 minutes.

Add Elution Buffer B, mix and incubate at 65°C for 10 minutes.

Place tube in magnetic separation rack. Let stand for 1 minute.

Carefully transfer supernatant to Elution Tube.

Pure Blood DNA
Procedure

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.
- **Always** vortex the Magnetic Bead Suspension before use.
- **Always** vortex the Proteinase K before use.
- Preheat the incubator(s) according to the protocol (37°C, 55°C or 65°C).
- Prepare a working concentration of the Solution WN by adding 24 mL of 96 - 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated 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.
- 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.
- **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.

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.
c. Add 300 µL of Lysis Buffer B 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.

**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.

f. Briefly spin the tube to collect any drops of liquid from the inside of the lid.
g. Add an equal volume of 96-100% Ethanol (100 µL of ethanol is added to every 100 µL of lysate) to the sample and mix well by vortexing for 10 seconds.
h. Add 40 µL of Magnetic Bead Suspension (vortex prior to use) to the lysate collected above.
i. Incubate at room temperature for 10 minutes. Occasionally invert the tube.
j. Briefly spin the tube to collect any drops of liquid from the inside of the lid.

2. Blood DNA Isolation

a. Assemble a magnetic separation rack and place the sample tube in the magnetic rack. Allow to sit for 1 minute.
b. Aspirate and discard supernatant without touching the magnetic beads.
c. Remove the sample tube from the magnetic rack and gently add 500 µL of Solution WN (ensure ethanol was added). Resuspend by vortexing or pipetting and incubate at room temperature for 1 minute.
d. Place the sample tube on the magnetic rack and allow to sit for 1 minute.
e. Aspirate and discard supernatant without touching the magnetic beads.
f. Remove the sample tube from the magnetic rack and gently add 500 µL of freshly prepared 70% ethanol. Resuspend by vortexing or pipetting and incubate at room temperature for 1 minute.
g. Place the sample tube on the magnetic rack and allow to sit for 1 minute.
h. Aspirate and discard supernatant without touching the magnetic beads.
i. Repeat Steps 2f - 2h for a second wash step.
Note: Remove as much of the 70% ethanol in the sample tube as possible by pipetting.

j. Incubate the open tube at 65°C for 5 minutes to dry the magnetic beads.
k. Remove the sample tube from the magnetic rack and add 100-200 µL of Elution Buffer B. Mix by vortexing and incubate at 65°C for 10 minutes.
l. Briefly vortex and place sample tube on the magnetic rack and allow to sit for 1 minute.
m. Carefully transfer the elution to a fresh 1.7 mL elution tube (provided) without touching the magnetic beads. 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 10⁷ 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

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution and Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnetic beads were accidently pipetted up with the supernatant.</td>
<td>The pipette tip was placed too close to the magnetic beads while pipetting</td>
<td>Return the magnetic beads and the supernatant back into the sample tube. Mix well, and place the tube back onto the magnetic separation rack for the specified time. Carefully remove the supernatant without touching the magnetic beads.</td>
</tr>
<tr>
<td>The yield of genomic DNA is low</td>
<td>Incomplete lysis of cells</td>
<td>Ensure that correct volume of Lysis Buffer B was added to blood sample. Also increase incubation time up to 15 minutes at 55°C.</td>
</tr>
<tr>
<td>DNA does not perform well in downstream applications.</td>
<td>DNA was not washed with 70% Ethanol</td>
<td>Traces of salt from the binding step may remain in the sample if the magnetic beads are not washed with 70% Ethanol. Salt may interfere with downstream applications, and thus must be washed from the magnetic beads.</td>
</tr>
<tr>
<td></td>
<td>Ethanol carryover</td>
<td>Ensure that the drying step after the 70% ethanol wash steps is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.</td>
</tr>
<tr>
<td>RNA is present in eluted DNA.</td>
<td>RNA is coeluted with the DNA.</td>
<td>Carry out a digestion with RNase A on the elution if the RNase present will interfere with downstream applications. Refer to manufacturer’s instructions regarding amount of enzyme to use, optimal incubation time and temperature.</td>
</tr>
<tr>
<td>Related Products</td>
<td>Product #</td>
<td></td>
</tr>
<tr>
<td>------------------------------------------------------</td>
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<tr>
<td>Blood Genomic DNA Isolation Mini Kit</td>
<td>46300</td>
<td></td>
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<tr>
<td>Dried Blood Spot Genomic DNA Isolation Kit</td>
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<tr>
<td>HighRanger 1kb DNA Ladder</td>
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</tr>
<tr>
<td>UltraRanger 1kb DNA Ladder</td>
<td>12100</td>
<td></td>
</tr>
</tbody>
</table>

**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.