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Blood DNA Isolation 96-Well Kit (Magnetic Bead System) Product Insert

Product # 62600

Norgen's Blood Genomic DNA Isolation 96-Well Kit (Magnetic Bead System) is designed for the rapid and high-throughput preparation of genomic DNA from up to 200 μL of whole blood from various species, including human. Purification is based on magnetic beads as the separation matrix. Norgen's magnetic beads bind DNA under optimized salt concentrations and release 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 Blood DNA Isolation 96-Well Kit (Magnetic Bead System) also can be integrated with a robotic automation system.

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 96-Well 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. Ethanol is then added to the lysate, and it is then transferred into a well of the 96-well plate. Magnetic Bead Suspension is 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

Kit Specifications	
Number of Preps	192
Maximum Blood Input	200 µL
Average Yield (200 µL of blood)*	4-12 µg
Average Purity (OD260/280)	1.7-1.9
Time to Complete 96 Purifications	50 minutes (hands-on time)

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

Advantages

- Fast, reproducible and easy processing using a Magnetic bead system
- High through put and compatible with automation robotic system
- Isolate high quality genomic DNA
- Recovered genomic DNA is compatible with various downstream applications

Kit Components

Component	Product # 62600 (192 samples)
Lysis Buffer B	1 x 40 mL 1 x 20 mL
Proteinase K in Storage Buffer	4 mL
Solution WN	55 mL
Elution Buffer B	1 x 30 mL 1 x 15 mL
Magnetic Bead Suspension	8.5 mL
96-Well Plate	2
96-Well Elution Plate	2
Adhesive Tape	2
Product Insert	1

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*.

Lysis Buffer B and **Solution WN** contain 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 saliva.

Customer-Supplied Reagents and Equipment

- Magnetic Bead 96-Well Separation Plate
- Multi-channel micropipettors
- Microcentrifuge tube
- 70% ethanol (prepare fresh)
- 96-100% ethanol
- Temperature adjustable (37°C, 55°C and 65°C) incubator
- 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 96-Well 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.

Transfer ↓

Add Magnetic Bead Suspension.
Mix and incubate for 5 minutes.

↓

Place onto magnetic plate.
Let stand for 1 minute.

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

Place onto magnetic plate..
Let stand 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 onto magnetic plate.
Let stand for 1 minute.

Carefully transfer supernatant
to Elution Plate. ↓

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 (37ºC, 55°C or 65°C).Prepare a working concentration of the **Solution WN** by adding 73 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 128 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 1.7×10^7 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 20,000 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. Transfer lysate into the 96-Well Plate, and add 40 μL of **Magnetic Bead Suspension** (vortex prior to use) to the lysate collected above. Mix by gentle pipetting.
i. Incubate at room temperature for 10 minutes. Occasionally vortex the 96-Well Plate.
j. Place the 96-Well Plate on the magnetic plate. Allow to sit for 1 minute.
k. Aspirate and discard supernatant without touching the magnetic beads.
l. Remove the 96-Well Plate from the magnetic plate, and gently add 500 μL of **Solution WN** (ensure ethanol was added). Resuspend by pipetting and incubate at room temperature for 1 minute.
m. Place the 96-Well Plate on the magnetic plate and allow to sit for 1 minute.
n. Aspirate and discard supernatant without touching the magnetic beads.
o. Remove the 96-Well Plate from the magnetic plate, and gently add 500 μL of freshly prepared **70% ethanol**. Resuspend by pipetting and incubate at room temperature for 1 minute.
p. Place the 96-Well Plate on the magnetic plate, and allow to sit for 1 minute.
q. Aspirate and discard supernatant without touching the magnetic beads.
r. Repeat **Steps 2o – 2q** for a second wash step.

Note: Remove as much of the 70% ethanol in the sample 96-Well Plate as possible by pipetting.

s. Incubate the 96-Well Plate at 65˚C for 5 minutes to dry the magnetic beads.
t. Add 100-200 µL of **Elution Buffer B**. Mix by gentle pipetting and incubate at 65˚C for 10 minutes.

u. Briefly vortex and place the 96-Well Plate on the magnetic plate and allow to sit for 1 minute.
v. Carefully transfer the elution to a 96-Well Elution Plate (provided) without touching the magnetic beads. The purified DNA sample may be stored at 4°C for a few days. The provided adhesive tape can be used for storage of the DNA. 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 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 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

Problem	Possible Cause	Solution and Explanation
Magnetic beads were accidently pipetted up with the supernatant.	The pipette tip was placed too close to the magnetic beads while pipetting	Return the magnetic beads and the supernatant back into the sample well. Mix well, and place the plate back onto the magnetic separation plate for the specified time. Carefully remove the supernatant without touching the magnetic beads.
The yield of genomic DNA is low	Incomplete lysis of cells	Ensure that correct volume of **Lysis Buffer B** was added to blood sample. Also increase incubation time up to 15 minutes at 55°C.
	Amount of magnetic beads added was not sufficient	Ensure that the magnetic bead suspension is mixed well prior to use to avoid any inconsistency in DNA isolation.
DNA does not perform well in downstream applications.	DNA was not washed with 70% ethanol	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.
	Ethanol carryover	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.
RNA is present in eluted DNA.	RNA is coeluted with the DNA.	Carry out a digestion with RNase A on the elution if the RNA present will interfere with downstream applications. Refer to manufacturer's instructions regarding amount of enzyme to use, optimal incubation time and temperature.

Related Products	Product #
Blood DNA Isolation Kit (Magnetic Bead System)	59800
Blood Genomic DNA Isolation Mini Kit	46300
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 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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