

Dried Blood Spot DNA Isolation Kit (Magnetic Bead System) – 50 Preps

Product # 76300 **Product Insert**

Norgen's Dried Blood Spot DNA Isolation (Magnetic Bead System) is designed for the rapid preparation of genomic DNA from Dried Blood Spot. 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 dried 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. Purification is based on a magnetic bead system, which replaces centrifuge steps and requires minimal laboratory equipment.

Norgen's Purification Technology

Purification is based on the use of magnetic beads that selectively bind genomic DNA under optimized binding conditions. Norgen's Dried Blood Spot DNA Isolation (Magnetic Bead System) kit is specifically designed to efficiently purify genomic DNA from dried blood spot samples. The magnetic bead-based chemistry enables the selective isolation of DNA while minimizing the co-purification of proteins and other cellular contaminants. Following binding, impurities are removed through wash steps, and the purified DNA is subsequently eluted under low-salt conditions. The resulting genomic DNA is of high purity and integrity and is suitable for a wide range of downstream applications including real-time PCR, next-generation sequencing, and microarray analysis.

Kit Components

Component	Product #76300 (50 samples)
Lysis Buffer B	20 mL
Proteinase K in Storage Buffer	1.2 mL
Magnetic Beads A	2.2 mL
Solution WN	18 mL
Elution Buffer B	8 mL
Elution Tubes	50 (2 x 25)
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Advantages

- Isolate high-quality genomic DNA, free from RNA contamination.
- Fast, reproducible and easy processing using a magnetic bead system.
- Purified DNA is suitable for a variety of downstream applications, including qPCR or NGS sequencing.

Specifications

Kit Specifications	
Number of Preps	50
Dried Blood Spots Input	Up to 6 x 3 mm (1/8 inch) diameter punches
Average Yield	150 ng*
Time to Complete 50 Purifications (Manual)	40 minutes (Hands-On Time)

* Average DNA yield will vary depending on the sample.

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. This kit is stable for 2 years after the date of shipment. The kit contains a ready-to-use Proteinase K, which is dissolved in a specially prepared storage buffer.

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 Safety Data Sheets (SDSs). These are available as convenient PDF files online at www.norgenbiotek.com.

Lysis Buffer B and **Wash Solution WN** contains 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.

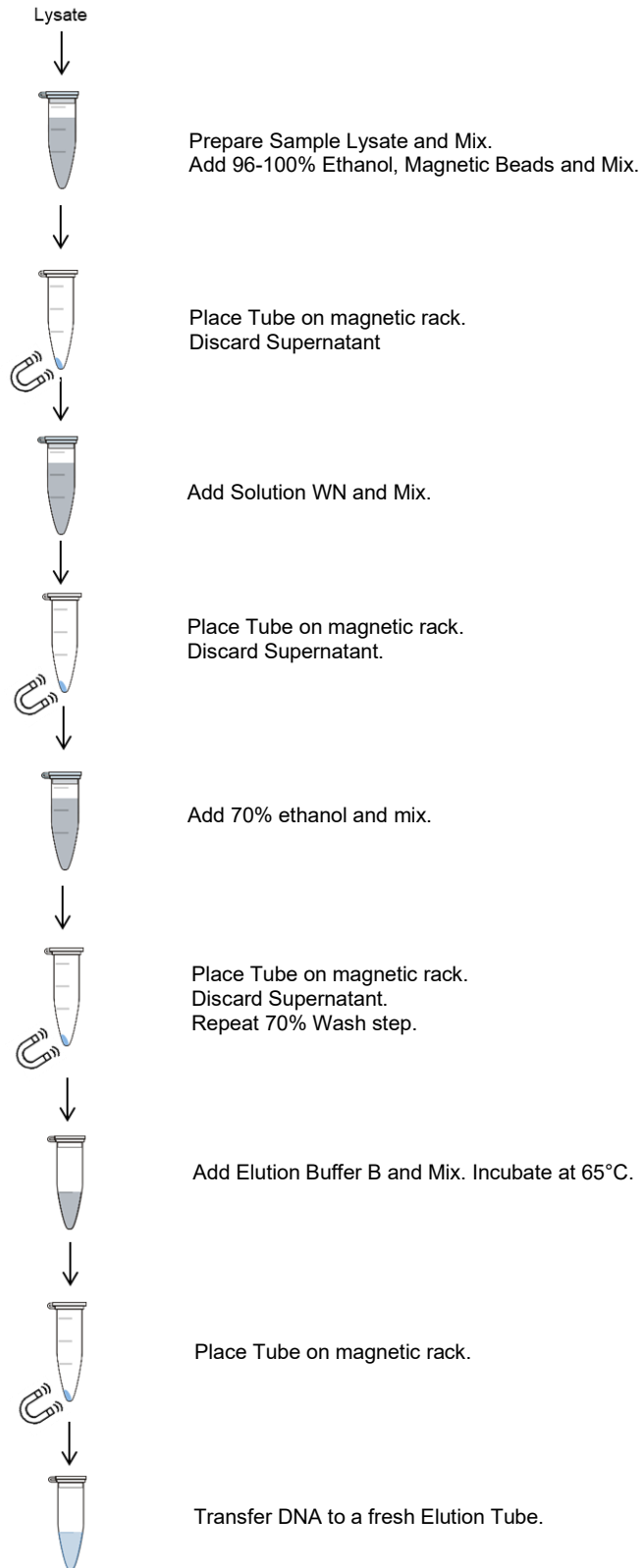
Blood from all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with these samples.

Customer-Supplied Reagents and Equipment

- Magnetic bead separation rack
- 1X PBS
- Micropipettors
- Microcentrifuge tube
- 70% ethanol (prepare fresh)
- 96 – 100% ethanol
- Temperature adjustable (55°C, 65°C) incubator(s)
- Nuclease-free water
- Orbital Shaker (Plates) or Tube Revolver Rotator (Tubes)
- RNase A (optional)

Flow Chart

Procedure for purifying DNA using Norgen's Dried Blood Spot DNA Isolation Kit (Magnetic Bead System)



Procedure

Notes prior to use:

- The procedure provides information and steps to follow to extract DNA using a Manual method.
- 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 Beads A** and **Proteinase K in Storage Buffer** before use.
- Preheat the incubator(s) according to the protocol (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.

Section 1. Dried Blood Spot DNA Isolation Using Manual Method

- 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 (not provided).
Note: Up to 6 x 3 mm diameter circles can be used to obtain higher DNA yield.
- b) Add 200 µL of the 1X PBS and vortex for 10 seconds.
- c) Add 20 µL of Proteinase K in Storage Buffer to a microcentrifuge tube.
- d) Add 300 µL of **Lysis Buffer B** to the tube and mix well by vortexing for 10 seconds.
- e) Briefly spin the tube to collect any drops of liquid from the inside of the lid. Incubate at 55°C for 10 minutes.
- f) Centrifuge the tube for 1 minute at 14,000 × g. Carefully transfer the clear supernatant to a new, clean microcentrifuge tube, avoiding any paper debris or precipitate.
Note: Do not transfer the remaining filter paper or collection card debris to the tube, transfer only the clear lysate.
- g) Add 500 µL of **96-100% ethanol** and 40 µL of **Magnetic Beads A** (vortex prior to use) to the lysate.
- h) Rotate the tubes on a tube revolver rotator at 10 RPM at room temperature for 5 minutes. In case the tube revolver rotator is not available, incubate the tubes at room temperature for 5 minutes and occasionally vortex the tube.
- i) Place the sample tube on the magnetic rack and allow to sit for 1 minute. Incubate for more time if the solution is not clear.
- j) Aspirate and discard supernatant without touching the magnetic beads.
- k) Remove the sample tube from the magnetic rack and gently add 500 µL of **Solution WN (ensure ethanol was added)**.
- l) Rotate the tubes on a tube revolver rotator at 10 RPM at room temperature for 2 minutes. In case the tube revolver rotator is not available, incubate the tubes at room temperature for 2 minutes and gently vortex the tube, occasionally.
- m) Place the sample tube on the magnetic rack and allow to sit for 1 minute. Incubate for more time if the solution is not clear.
- n) Aspirate and discard supernatant without touching the magnetic beads.
- o) Remove the sample tube from the magnetic rack and gently add 750 µL of freshly prepared **70% ethanol**.
- p) Rotate the tubes on a tube revolver rotator at 10 RPM at room temperature for 2 minutes. In case the tube revolver rotator is not available, incubate the tubes at room temperature for 2 minutes and gently vortex the tube, occasionally.
- q) Place the sample tube on the magnetic rack and allow to sit for 1 minute. Incubate for more time if the solution is not clear.
- r) Aspirate and discard the supernatant without touching the magnetic beads.

- s) Repeat **Steps o to r from this section** for a second wash step.
- t) Remove as much of the wash solution as possible and air-dry the beads at room temperature for 5 to 10 minutes. (Do not let the magnetic beads to over dry as this may affect the DNA yield).
- u) Remove the tube from the magnetic rack and add 50-100 μ L of **Elution Solution B**. Mix by pipetting and incubate at 65°C for 10 minutes.
- v) Incubate the tubes at room temperature for 2 minutes and occasionally gently mixing by vortexing the tube. Briefly spin down the tubes to bring the contents at the bottom of the tube.
- w) Place the sample tubes on the magnetic rack. Allow to sit for 1 minute. Incubate for more time if the solution is not clear.
- x) Carefully transfer the elution to a fresh Elution Tube (provided) without touching the magnetic beads.
- y) 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
Magnetic beads were accidentally 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 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.
The yield of genomic DNA is low	Incomplete lysis of cells	Ensure that correct volume of Lysis Buffer B was added to DBS 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.

Related Products	Product #
Blood DNA Isolation Kit 96-Well Kit (Magnetic Bead System)	62600
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 9:00 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.

3430 Schmon Parkway, Thorold, ON Canada L2V 4Y6
Phone: (905) 227-8848
Fax: (905) 227-1061
Toll Free in North America: 1-866-667-4362