NORGEN BIOTEK CORPORATION

3430 Schmon Parkway
Thorold, ON, Canada L2V 4Y6
Phone: 866-667-4362 • (905) 227-8848
Fax: (905) 227-1061
Email: techsupport@norgenbiotek.com

Blood DNA Purification Kit Plus – 30mL

Product Insert

Product #52400

Norgen's Blood DNA Purification Kit Plus – 30 mL provides a fast and simple procedure for purifying high molecular weight genomic DNA from up to 10 mL of blood. The kit allows for the isolation of genomic DNA from the blood of various species, including humans. Typical yields of genomic DNA will vary depending on the cell density of the blood sample. Preparation time for a single sample is about 60 minutes. Blood genomic DNA purified using Norgen's kit is of the highest quality, and is compatible with a number of downstream applications including PCR, Southern Blot analysis, sequencing and microarray analysis.

Purification is based on the lysis of red blood cells (RBC) using the Norgen's **RBC Lysis Solution** followed by precipitating the white blood cells (WBC). Norgen's **Cell Lysis Solution** and **Proteinase K** are then added to the WBC pellet to lyse the cells and remove proteins. Genomic DNA is then recovered by alcohol precipitation and resuspended in the **DNA Rehydration Solution**. The size of the purified blood genomic DNA is up to 200 kpb with a typical A_{260}/A_{280} ratio of >1.7.

Specifications

Kit Specifications	
Minimum blood input	0.3 mL
Maximum blood input	10 mL
Average Yield from 1 mL of whole blood (7×10^6 white blood cells)	24 µg*
DNA size	Up to 200 kbp
Average purity (OD260/280)	>1.7
Time to Complete 10 Purifications	45-60 minutes (+ DNA rehydration)

*Yield will vary depending on the type of blood processed

Advantages

- Fast and simple processing
- DNA can be isolated and detected from as little as 300 µL of blood
- Isolate high quality and high molecular weight genomic DNA
- Recovered genomic DNA is compatible with various downstream applications

Kit Components

Component	Product #52400
RBC Lysis Solution	100 mL
Cell Lysis Solution	40 mL
Proteinase K	2.5 mL
DNA Rehydration Solution	12 mL
Product Insert	1

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. The kit contains a ready-touse Proteinase K solution, which is dissolved in a specially prepared storage buffer. The Proteinase K is stable for up to 1 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*.

The **Cell Lysis Solution** 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

- Benchtop microcentrifuge (variable speed, up 14,000 x g) or swing bucket centrifuge (≥ 2,000 x g)
- Micropipettors and pipette tips
- 1.5 mL sterile microcentrifuge tubes, 15 mL centrifuge tubes or 50 mL centrifuge tubes
- Isopropanol
- 70% ethanol (does not contain other substances such as methanol or methylethylketone)
- 56°C waterbath or incubator
- 65°C incubator
- Crushed ice
- 37°C incubator (for blood containing Gram positive bacterial pathogens)
- Lysozyme (for blood containing Gram positive bacterial pathogens)

Procedure

Please check your microcentrifuge or centrifuge 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:

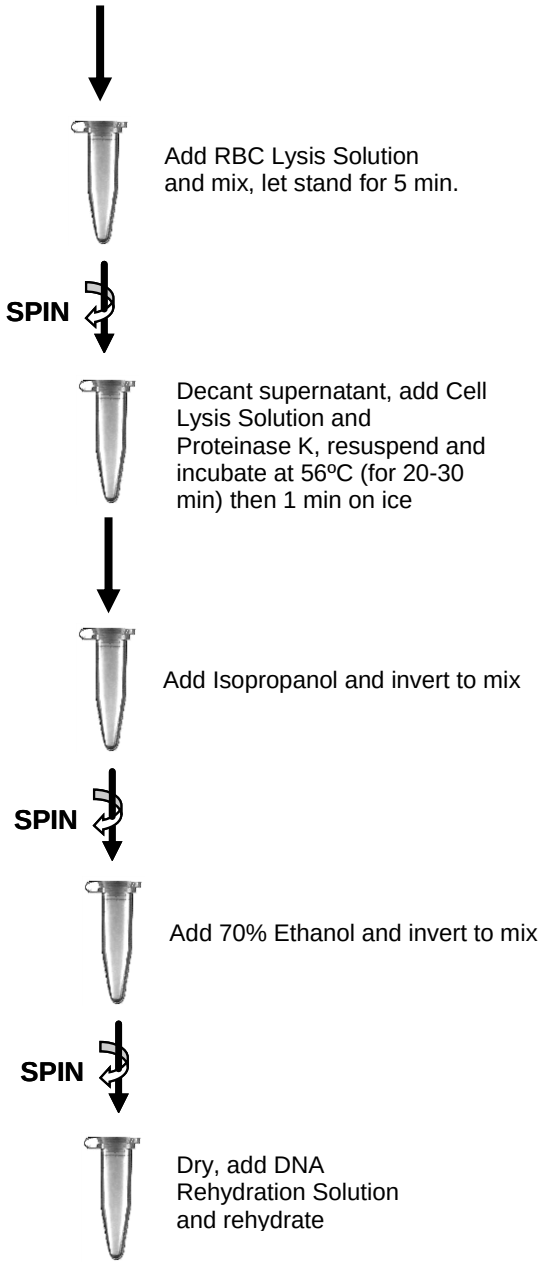
$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\,(r)}}$$

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 Genomic DNA using Norgen's Blood DNA Purification Kit Plus – 30 mL

Desired volume of Blood in the appropriate tube size



Pure Blood Genomic DNA

Volumes Required for Scaling Whole Blood Purification Protocols to Process Different Blood Input Volumes

*Blood volume (µL)	300	3,000	10,000
†Tube size (mL)	1.5	15	50
RBC Lysis Solution (µL)	900	9,000	30,000
Cell Lysis Solution (µL)	300	3,000	10,000
Proteinase K (µL)	22.5	225	750
Isopropanol (µL)	375	3,500	11,000
70% ethanol (µL)	900	9,000	30,000
‡DNA Rehydration Solution (µL)	100	300	1,000

*To isolate DNA from different volumes of blood than shown above, use kit solution volumes that are proportional to the starting blood volume.
†Use 50 mL centrifuge tube if the starting blood volume exceeds 3.5mL.
‡Lower volumes can be used to obtain higher DNA concentration, however longer rehydration time may be required.

Notes prior to use:

- **Blood Sample:** Fresh or frozen whole blood samples treated with the appropriate anticoagulant (EDTA, citrate or heparin) may be used. Better yield is obtained from fresh blood and EDTA is preferred for high molecular weight genomic DNA. Blood can be collected on the desired anticoagulant and stored at -80 before genomic DNA purification.
- 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.
- Thaw frozen blood samples quickly at 37°C with gentle agitation and keep on ice before starting the purification procedures.
- Vortex **Proteinase K** before use.
- Prepare the required volume of 70% ethanol.
- Preheat water bath or incubator to 56°C.
- Preheat 65°C incubator for the final rehydration step.
- **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.
- Vigorous handling of DNA during the different steps of the protocol can shear the DNA. Avoid vigorous vortexing and use gentle pipetting in all of the mixing and resuspension steps if higher molecular weight DNA is required.

A. Purification Procedure for 300 µL Blood Sample

1. Add 900 µL of **RBC Lysis Solution** to a 1.5 mL microcentrifuge tube.
2. Add 300 µL of whole blood, and mix by inverting 10 times.
3. Incubate at room temperature for 3 minutes, and invert once during the incubation.
4. Centrifuge for 5 minutes at 2,000 x g to pellet white blood cells.
5. Discard the supernatant by pipetting, leaving approximately 50 µL of residual liquid. Make sure not to disturb the white blood cells pellet.

Note: For DNA isolation from **blood containing Gram positive bacterial pathogens**, add 5 µL of lysozyme (not provided) and mix well by pipetting. Incubate at 37°C for 1 hour (NOTE: incubation times may fluctuate between 0.5 and 2 hours depending on the bacterial strain being lysed). After incubation, proceed to **Step 6**.

6. Add 300 µL of **Cell Lysis Solution**.
7. Add 22.5 µL of **Proteinase K** (vortex Proteinase K before use).
8. Pipette gently to resuspend the pellet in the solutions. Make sure that the pellet is completely dispersed.
9. Incubate at 56ºC for 20 minutes.
10. Place the lysate tube in ice for 1 minute.
11. Add 375 µL of Isopropanol and invert 50 times to mix. DNA threads or clump may be visible after mixing.
12. Centrifuge for 1 minute at 14,000 x g. A white pellet of DNA may be visible.
13. Carefully discard the supernatant without disturbing the pellet. Invert the tube on clean absorbent paper for 1 minute to drain residual isopropanol.
14. Add 900 µL of 70% ethanol, and invert 10 times.
15. Centrifuge for 1 minute at 14,000 x g.
16. Carefully discard the supernatant. Invert the tube upside down on clean absorbent paper for 5 minutes to drain residual ethanol and air dry. Handle the tube carefully as the DNA pellet can dislodge. Make sure not to over dry the DNA.
17. Add 100 µL of **DNA Rehydration Solution**, and pipette gently to resuspend DNA.

    Note: For lower or higher DNA concentrations, the volume of DNA Rehydration Solution can be increased or decreased, respectively.

18. Incubate at 65ºC for 5 minutes to completely rehydrate DNA. Ensure that the DNA is completely rehydrated. Gentle pipetting as well as overnight incubation at room temperature may be used.

    Note: The prepared high yield DNA might be viscous due to the high concentration and molecular weight, which can affect DNA analysis. It is recommended to use a larger DNA Rehydration Solution volume and/or longer rehydration time with gentle pipetting to ensure that no aggregates are formed.

B. Purification Procedure for 3 mL Blood Sample

1. Add 9 mL of **RBC Lysis Solution** to a 15 mL centrifuge tube.
2. Add 3 mL of whole blood, and mix by inverting 10 times.
3. Incubate at room temperature for 5 minutes, and invert at least once during the incubation.
4. Centrifuge for 5 minutes at 2,000 x g to pellet white blood cells.
5. Carefully discard the supernatant by pipetting or pouring, leaving approximately 200 µL of residual liquid. Make sure not to disturb the white blood cells pellet.

   Note: For DNA isolation from **blood containing Gram positive bacterial pathogens**, add 20 µL of lysozyme (not provided) and mix well by pipetting. Incubate at 37°C for 1 hour (NOTE: incubation times may fluctuate between 0.5 and 2 hours depending on the bacterial strain being lysed). After incubation, proceed to step **6**.

6. Add 3 mL of **Cell Lysis Solution**.
7. Add 225 µL of **Proteinase K** (vortex Proteinase K before use).
8. Pipette gently to resuspend the pellet into the added solutions. Make sure that the pellet is completely dispersed.
9. Incubate at 56ºC for 25 minutes.
10. Place the lysate tube in ice for 2 minutes.
11. Add 3.5 mL of Isopropanol and invert 50 times to mix. DNA threads or clump may be visible after mixing.
12. Centrifuge for 3 minutes at ≥ 2,000 x g. A white pellet of DNA may be visible.
13. Carefully discard the supernatant without disturbing the pellet. Invert the tube on clean absorbent paper for 1 minute to drain residual isopropanol.
14. Add 9 mL of 70% ethanol, and invert 10 times.

15. Centrifuge for 1 minute at ≥ 2,000 x g.
16. Carefully discard the supernatant. Invert the tube upside down on clean absorbent paper for 10 minutes to drain residual ethanol and air dry. Handle the tube carefully as the DNA pellet can dislodge. Make sure not to over dry the DNA.
17. Add 300 µL of **DNA Rehydration Solution**, and pipette gently to resuspend DNA.

    Note: For lower or higher DNA concentrations, the volume of DNA Rehydration Solution can be increased or decreased, respectively.

18. Incubate at 65ºC for 60 minutes to completely rehydrate DNA. Ensure that the DNA is completely rehydrated. Gentle pipetting as well as overnight incubation at room temperature may be used.

    Note: The prepared high yield DNA might be viscous due to the high concentration and molecular weight, which can affect DNA analysis. It is recommended to use a larger DNA Rehydration Solution volume and/or longer rehydration time with gentle pipetting to ensure that no aggregates are formed.

C. Purification Procedure for 10 mL Blood Sample

1. Add 30 mL of **RBC Lysis Solution** to a 50 mL centrifuge tube.
2. Add 10 mL of whole blood, and mix by inverting 10 times.
3. Incubate at room temperature for 5 minutes, and invert at least once during the incubation.
4. Centrifuge for 5 minutes at 2,000 x g to pellet white blood cells.
5. Carefully discard the supernatant by pipetting or pouring, leaving about 200 µL of residual liquid. Make sure not to disturb the white blood cells pellet.

   Note: For DNA isolation from **blood containing Gram positive bacterial pathogens**, add 50 µL of lysozyme (not provided) and mix well by pipetting. Incubate at 37°C for 1 hour (NOTE: incubation times may fluctuate between 0.5 and 2 hours depending on the bacterial strain being lysed). After incubation, proceed to step **6**.

6. Add 10 mL of **Cell Lysis Solution**.
7. Add 750 µL of **Proteinase K** (vortex Proteinase K before use).
8. Pipette gently to resuspend the pellet into the added solutions. Make sure that the pellet is completely dispersed.
9. Incubate at 56ºC for 30 minutes.
10. Place the lysate tube in ice for 3 minutes.
11. Add 11 mL of Isopropanol and invert 50 times to mix. DNA threads or clump may be visible after mixing.
12. Centrifuge for 5 minutes at ≥ 2,000 x g. A white pellet of DNA may be visible.
13. Carefully discard the supernatant without disturbing the pellet. Invert the tube on a clean absorbent paper for 1 minute to drain residual isopropanol.
14. Add 30 mL of 70% ethanol, and invert 10 times.
15. Centrifuge for 2 minutes at ≥ 2,000 x g.
16. Carefully discard the supernatant. Invert the tube upside down on clean absorbent paper for 10 minutes to drain residual ethanol and air dry. Handle the tube carefully as the DNA pellet can dislodge. Make sure not to over dry the DNA.
17. Add 1 mL of **DNA Rehydration Solution**, pipette gently to resuspend DNA.

    Note: For lower or higher DNA concentrations, the volume of DNA Rehydration Solution can be increased or decreased, respectively.

18. Incubate at 65ºC for 60 minutes to completely rehydrate DNA. Ensure that the DNA is completely rehydrated. Gentle pipetting as well as overnight incubation at room temperature may be used.

Note: The prepared high yield DNA might be viscous due to the high concentration and molecular weight, which can affect DNA analysis. It is recommended to use a larger DNA Rehydration Solution volume and/or longer rehydration time with gentle pipetting to ensure that no aggregates are formed.

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 or at -80°C for archival sample storage.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Cells are not completely lysed	Too many cells were used	The protocol is optimized for a cell density of 7 x 10^6 white blood cells per 1 mL of blood. Exceeding the recommended input will increase the viscosity and the cells will clump due to incomplete lysis. More lysis solution will be required. To avoid this, cell counting of the blood sample is recommended.
	The lysate mixture was not completely mixed after the addition of the Cell Lysis Solution and Proteinase K	Make sure to completely mix the lysate by gentle pipetting. No cell clumps should be visible after mixing and the cell pellet should be completely dislodged.
Samples are not completely rehydrated	Samples were not resuspended after adding the DNA Rehydration Solution	Make sure to pipette well to resuspend DNA into the DNA Rehydration Solution. Avoid vigorous vortexing if higher molecular weight DNA is required
	DNA pellet over drying	Do not exceed the recommended air drying time. Over drying will slow down the rehydration step and more incubation time at 65 ºC (up to 1 hour) may be required.
	Samples were not incubated at 65ºC after resuspending in the DNA Rehydration Solution	Make sure to incubate the sample at 65ºC for the recommended time. Up to 1 hour incubation at 65ºC can be used if the sample is not completely rehydrated.
	Protein carry over in the purified DNA	Make sure not to exceed the recommended input amount of cells.
Low yield	Low input	Count cells to ensure that your sample has a high enough amount of starting cells.
	Incomplete lysis	Exceeding the recommended input will increase the viscosity and cells will clump due to incomplete lysis, resulting in a lower yield. To avoid this, cell counting of the blood sample is recommended.
	Incomplete rehydration	Do not over dry the DNA pellet before adding the DNA Rehydration Solution. Mix well to resuspend the DNA before incubating at 65ºC for the specified time. Up to 1 hour incubation at 65ºC may be used to ensure complete rehydration.
High A_{260}/A_{280}	RNA contamination	RNase treatment of purified sample can be carried out to remove residual RNA.
DNA size is less than 50 kbp	DNA degradation	Blood samples should be stored at 4ºC for few days or at -20ºC if storage exceeds 5 days. Storage at -80°C is recommended for archival sample storage.
	DNA shearing	Vigorous handling of DNA during the different steps of the protocol can shear the DNA. Avoid vigorous vortexing and use gentle pipetting in all of the mixing and resuspension steps.

Related Products	Product #
Blood Genomic DNA Isolation Micro Kit	52100
Blood Genomic DNA Isolation Mini Kit	46300
Blood Genomic DNA Isolation Midi Kit	51400
Blood Genomic DNA Isolation Maxi Kit	31200
Dried Blood Spot Genomic DNA Isolation Kit	36000

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.

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

