

PAXgene[®]

Blood DNA Kit Handbook

For isolation of genomic DNA from 8.5 ml human whole blood

Important: To be used only in conjunction with PAXgene Blood DNA Tubes

For research use only

Not for use in diagnostic procedures

December 2015

 PreAnalytiX[™]
A QIAGEN / BD Company

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Kit Contents

PAXgene Blood DNA Kit (25)	761133
Number of preps	25
Processing Tubes containing 25 ml Buffer BG1 (lysis buffer)	25
Buffer BG2 (wash buffer)	140 ml
Buffer BG3* (digestion buffer)	140 ml
Buffer BG4 (resuspension buffer)	50 ml
PreAnalytiX® Protease	1 vial†
Handbook	1

* Contains chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 6 for safety information.

† Resuspension volume 1.4 ml

Storage

All buffers and reagents can be stored dry at room temperature (15–25°C) for up to 9 months.

Lyophilized PreAnalytiX Protease can be stored at room temperature (15–25°C) for up to 6 months without reduction in performance. For storage longer than 6 months or if ambient temperatures frequently exceed 25°C, PreAnalytiX Protease should be stored dry at 2–8°C.

Reconstituted PreAnalytiX Protease is stable for 2 months when stored at 2–8°C. Incubating the PreAnalytiX Protease stock solution at room temperature for prolonged periods should be avoided. Storage at –30 to –15°C will prolong its life, but repeated freezing and thawing should be avoided. Dividing the solution into aliquots and storage at –30 to –15°C is recommended.

Product Use Limitations

For research use only. Not for use in diagnostic procedures.

Product Warranty and Satisfaction Guarantee

PreAnalytiX guarantees the performance of all products in the manner described in our literature. The purchaser must determine the suitability of the product for its particular use.

PreAnalytiX products are manufactured for PreAnalytiX (Hombrechtikon, CH) by QIAGEN or BD and are distributed for PreAnalytiX by QIAGEN.

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A copy of PreAnalytiX terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see last page or visit www.qiagen.com).

Technical Assistance

Technical assistance with PreAnalytiX products is provided by QIAGEN, the distributor for PreAnalytiX. The Technical Service Departments at QIAGEN are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology. If you have any questions or experience any difficulties regarding the PAXgene Blood DNA System, please do not hesitate to contact one of the QIAGEN Technical Services Departments listed on page 19.

PreAnalytiX customers are a major source for information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at PreAnalytiX. We therefore encourage you to contact us through QIAGEN's Technical Service Departments if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see last page or visit www.qiagen.com).

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of PAXgene Blood DNA Kits is tested against predetermined specifications to ensure consistent product quality.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each PreAnalytiX kit and kit component.



CAUTION: DO NOT add bleach or acidic solutions directly to the sample preparation waste.

The sample-preparation waste contains guanidine hydrochloride from Buffer BG3, which can form highly reactive compounds when combined with bleach.

If liquid containing Buffer BG3 is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Introduction

Applications such as pharmacogenomic studies, gene expression studies, and SNP genotyping yield clinically important data. Use of standardized methods for sample collection and nucleic acid isolation for such studies is of increasing importance.

The PAXgene Blood DNA System is an integrated and standardized system for collection, transport, and storage of whole blood specimens and isolation of genomic DNA. Blood is collected into PAXgene Blood DNA Tubes, which contain a proprietary blend of reagents, optimized for isolation of high-quality genomic DNA. After transport and/or storage, DNA is isolated from the tubes using the PAXgene Blood DNA Kit. The purification is performed in a single processing tube to minimize the risks of sample mix-up and cross contamination. High yields of pure DNA are obtained that perform well in a wide range of downstream applications.

The PAXgene Blood DNA Kit principle and procedure

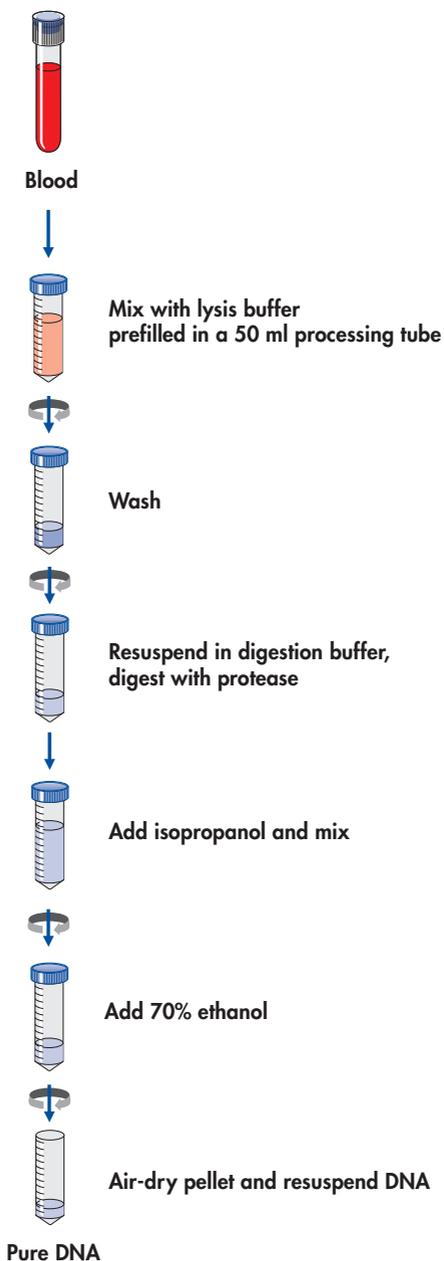
The PAXgene Blood DNA Kit allows isolation of total DNA from 8.5 ml of human whole blood collected in PAXgene Blood DNA Tubes. The isolation procedure is easy to perform, rapid, and results in high yields of pure, high-molecular-weight DNA. The procedure (see flowchart, page 8) starts with transfer of blood from PAXgene Blood DNA Tubes to processing tubes filled with cell lysis buffer. The solution is briefly mixed to lyse red and white blood cells. Cell nuclei and mitochondria are pelleted by centrifugation, washed, and resuspended in digestion buffer. Protein contaminants are removed by incubation with protease enzyme. DNA is precipitated in isopropanol, washed in 70% ethanol, dried, and resuspended in resuspension buffer.

The PAXgene Blood DNA System typically results in DNA yields of 150–500 µg DNA and A_{260}/A_{280} ratios of 1.7–1.9. However, yields depend on the number of nucleated cells present. Yields from different donors may vary widely, since white blood cell counts can differ as much as tenfold. The DNA is up to 200 kb in size, with fragments of 50–150 kb predominating.

The isolated DNA is ready for use in downstream applications, such as:

- PCR (including long-range, multiplex, and quantitative real-time PCR)
- Restriction digestion and Southern-blotting techniques
- SNP genotyping
- Pharmacogenomic studies

The PAXgene Blood DNA Procedure



Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- PAXgene Blood DNA Tubes (see ordering information, page 17)
- 100% isopropanol
- 70% (v/v) ethanol*
- Pipets and pipet tips
- Centrifuge capable of attaining 2500 x *g*, equipped with a swing-out rotor and buckets that accommodate 50 ml Processing Tubes
- Heating block or water bath capable of 65°C
- Vortex mixer

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

Important Notes

Storage of blood samples

Blood samples collected using PAXgene Blood DNA Tubes can be stored at 15–25°C for up to 14 days, at 2–8°C for up to 28 days,* or at –20°C for up to 3 months.

Freeze PAXgene Blood DNA Tubes upright in a wire rack or horizontally in a plastic bag. Do not freeze tubes upright in a Styrofoam® collection tray as this may cause the tubes to crack.

For long-term storage, freezing the samples at –70°C is recommended. If tubes are to be kept at temperatures below –20°C, freeze them first at –20°C for 24 hours, and then transfer them to –70°C or –80°C.

Thaw frozen PAXgene Blood DNA Tubes in a wire rack at ambient temperature (18–25°C) for approximately 2 hours or at 37°C in a water bath for approximately 15 minutes. Carefully invert the thawed PAXgene Blood DNA Tubes 10 times.

Note: Frozen PAXgene Blood DNA Tubes are subject to breakage upon impact. To reduce the risk of breakage during shipment, frozen tubes should be treated the same as glass tubes. Users must validate their own shipping protocol for PAXgene Blood DNA Tubes.

Optimization of centrifugation conditions

The centrifugation steps in the protocol are key to optimal nucleic acid recovery. A centrifuge with adjustable *g*-force is highly recommended for use with this protocol. If no such centrifuge is available, the *g*-force can be calculated as follows:

$$rcf = 11.18 \times r \times (rpm/1000)^2$$

where *rcf* is the relative centrifugal force (in *g*), *r* is the radius of the rotor in centimeters, and *rpm* is the speed of the centrifuge in revolutions per minute.

* The storage information for storage at 2–8°C is based on studies performed using small numbers of samples, and cannot be guaranteed to be statistically valid.

Protocol: Purification of Genomic DNA from Human Whole Blood Collected into PAXgene Blood DNA Tubes

The PAXgene Blood DNA Kit is used for purification of genomic DNA from 8.5 ml of human whole blood, collected in PAXgene Blood DNA Tubes (cat. no. 761115 [USA and Canada only], 761105 [Japan only] or 761125 [all other countries]). PAXgene Blood DNA Tubes and the PAXgene Blood DNA Kit are an integrated system for collection of whole blood and isolation of genomic DNA.

Important note before starting

- All centrifugation steps should be carried out at room temperature (15–25°C) in a swing-out rotor.

Things to do before starting

- Thaw frozen PAXgene Blood DNA Tubes in a wire rack at ambient temperature (18–25°C) for approximately 2 hours or at 37°C in a water bath for approximately 15 minutes. Carefully invert the thawed PAXgene Blood DNA Tubes 10 times before beginning the procedure.
- Heat a heating block or water bath to 65°C for use in steps 8 and 17.
- Add 1.4 ml Buffer BG4 (resuspension buffer) to lyophilized PreAnalytiX Protease. Dissolved PreAnalytiX Protease should be stored at 2–8°C or in aliquots at –20°C (see “Storage”, page 4).
- For every sample, mix 5 ml Buffer BG3 (digestion buffer) and 50 µl reconstituted PreAnalytiX Protease. For example, to process 10 samples, mix 50 ml Buffer BG3 with 500 µl PreAnalytiX Protease. The Buffer BG3–PreAnalytiX Protease mixture should be prepared immediately before the start of the procedure.

Procedure

1. **Pour all the blood from one PAXgene Blood DNA Tube into a Processing Tube containing 25 ml Buffer BG1. Close the tube. To avoid cracking the blue lids of the Processing Tubes, do not overtighten them. Tighten the lid only until the first sign of resistance is felt. Mix by inverting the tube 5 times.**

If the blood in the PAXgene Blood DNA Tube has separated into plasma and red blood cells, invert the tubes carefully 10 times to homogenize the sample.

2. **Centrifuge for 5 min at 2500 x g in a swing-out rotor.**
3. **Carefully discard the supernatant and place the tube in a rack.**
In rare cases the pellet may be loose, so pour slowly.
4. **Add 5 ml Buffer BG2, close the tube, and wash the pellet by vortexing vigorously for 5 s.**
5. **Centrifuge for 3 min at 2500 x g in a swing-out rotor.**

- 6. Carefully discard the supernatant and place the tube back in the rack.**

In rare cases the pellet may be loose, so pour slowly.
- 7. Add 5 ml Buffer BG3/PreAnalytiX Protease (see “Things to do before starting”), close the tube, and vortex for 20 s at high speed.**

Vortexing for 20 s is essential to dissolve the pellet completely. Shorter vortexing times may lead to incomplete resuspension of the pellet and reduced DNA yield or purity. After this step, samples can be stored for at least 7 days at 2–8°C. After storage, resume the procedure at step 8.
- 8. Place the tube in a heating block or water bath and incubate at 65°C for 10 min.**

The sample changes color from light red to light green, indicating that protein digestion has occurred.
- 9. Vortex again for 5 s at high speed.**
- 10. Add 5 ml isopropanol (100%) and mix by inverting the tube at least 20 times until the white DNA strands clump visibly together.**

Complete mixing with isopropanol is essential to precipitate the DNA and should be checked by inspection. Only tightly clumped DNA strands can be efficiently pelleted by centrifugation. Do not vortex as this might reduce DNA yield.
- 11. Centrifuge for 3 min at 2500 x g.**
- 12. Discard the supernatant and leave the tube inverted on a clean piece of absorbent paper for 1 min.**

In rare cases the pellet may be loose, so pour slowly. Inverting the tube onto absorbent paper minimizes backflow of isopropanol from the rim and sides of the tube onto the pellet.
- 13. Add 5 ml 70% (v/v) ethanol and vortex for 1 s at high speed.**
- 14. Centrifuge for 3 min at 2500 x g.**
- 15. Discard the supernatant and leave the tube inverted on a clean piece of absorbent paper for at least 5 min.**

In rare cases the pellet may be loose, so pour slowly. Inverting the tube onto absorbent paper minimizes backflow of ethanol from the rim and sides of the tube onto the pellet.
- 16. Carefully dab the tube onto absorbent paper to remove ethanol from the rim, and leave it inverted for a further 5 min to allow the DNA pellet to dry.**

Avoid overdrying the pellet, since overdried DNA is very difficult to dissolve.
- 17. Add 1 ml Buffer BG4 and dissolve the DNA by incubating for 1 h at 65°C in a heating block or water bath, followed by incubation overnight at room temperature.**

Highly concentrated, high-molecular-weight genomic DNA samples may not redissolve completely after an incubation of 1 h at 65°C, therefore an additional overnight incubation at room temperature is recommended.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see last page or visit www.qiagen.com).

Comments and suggestions

Low DNA yield

- | | |
|---|---|
| a) Reduced activity of protease | Ensure that the PreAnalytiX Protease was dissolved in the correct volume of Buffer BG4. |
| b) No DNA precipitate visible after addition of isopropanol | Ensure that the sample is completely mixed with isopropanol. Invert the tube at least 20 times after addition of isopropanol, until the strands of DNA clump together. |
| c) DNA was overdried | Avoid overdrying DNA pellet after removal of 70% (v/v) ethanol, as overdried genomic DNA is difficult to dissolve. At step 17, prolong the incubation time used to resuspend the DNA to 2 hours, then leave the solution overnight at room temperature. |
| d) DNA not completely dissolved | Ensure that the DNA is dissolved completely by incubating for 1 hour at 65°C, followed by incubation overnight at room temperature. If the DNA is still not fully dissolved, vortex at low speed for 5 seconds and incubate again for 10 minutes at 65°C. |

Low DNA purity

- | | |
|---|--|
| a) Pellet difficult to resuspend after addition of Buffer BG2 and vortexing for 5 seconds | Follow the centrifugation recommendations given in the protocol. In rare cases, it may be necessary to prolong the vortexing at step 4 or to facilitate resuspension by pipetting up and down. |
|---|--|

Comments and suggestions

- b) Jelly-like consistency of DNA pellet after isopropanol precipitation

Ensure that the sample was completely dissolved at step 7 by vortexing for 20 seconds at high speed. If the pellet does not dissolve completely, prolong vortexing at step 9 until the remaining particles are completely removed.

Ensure that the sample was completely mixed with isopropanol. Invert the tube at least 20 times after addition of isopropanol, until the strands of DNA clump together.

- c) Beige DNA pellet, or A_{260}/A_{280} ratio of the purified DNA is <1.65

Do not store blood samples for longer than 14 days at 15–25°C. For short-term storage (up to 4 weeks), store blood at 2–8°C. For long-term storage, freezing the samples at –70°C is recommended. If a –70°C freezer is not available, samples can also be stored at –20°C.

Purified DNA does not perform well in downstream enzymatic applications

- a) Final DNA solution contaminated with ethanol

Ensure that all the ethanol has evaporated before adding Buffer BG4. Leave the tube at step 15 inverted on a clean piece of absorbent paper for at least 5 minutes to minimize backflow of ethanol from the rim and sides of the tube onto the pellet.

- b) Wrong amount of DNA used in the downstream application

Amplification reactions are often inhibited by excess DNA. Reduce the amount of DNA used — 20–50 ng is usually sufficient for PCR.

Appendix: Analyzing DNA

Determination of concentration, yield, and purity

DNA yield is determined from the concentration of DNA in the eluate, measured by absorbance at 260 nm. Absorbance readings at 260 nm should fall between 0.1 and 1.0 to be accurate. Use Buffer BG4 (as appropriate) to dilute samples and to calibrate the spectrophotometer. Measure the absorbance at 260 and 280 nm, or scan absorbance from 220–320 nm (a scan will show if there are other factors affecting absorbance at 260 nm).

Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. Pure DNA has an A_{260}/A_{280} ratio of 1.7–1.9. DNA purified by the PAXgene Blood DNA System procedure is free of protein and other contaminants that can inhibit PCR or other enzymatic reactions. The purified DNA can be used immediately or stored in Buffer BG4 at -20°C for later use.

Determination of DNA length

The length of the purified DNA can be determined by comparison with DNA markers of known size, using pulsed-field agarose gel electrophoresis (PFGE) and ethidium bromide staining. Load 1–5 μg DNA per well. Standard PFGE conditions are as follows: 1% agarose gel in 0.5x TBE electrophoresis buffer (45 mM Tris-borate; 1 mM EDTA, pH 8.0); switch intervals, 5–40 seconds; run time, 17 hours; voltage, 170 V. The expected size of genomic DNA purified using the PAXgene Blood DNA System is up to 200 kb (predominantly 50–150 kb).

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN and PreAnalytiX products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Ordering Information

Product	Contents	Cat. no.
Products that can be ordered from QIAGEN		
PAXgene Blood DNA System — a standardized system for collection and transport of blood and isolation of genomic DNA		
PAXgene Blood DNA Tubes (100)	100 Blood Collection Tubes. To be used in conjunction with the PAXgene Blood DNA Kit (25).	761115* 761105† 761125‡
PAXgene Blood DNA Kit (25)	25 Processing Tubes filled with Lysis Buffer, Buffers and Reagents. To be used in conjunction with PAXgene Blood DNA Tubes.	761133
Products that can be ordered from BD and BD authorized distributors[§]		
Blood Collection Set	BD Vacutainer® Safety-Lok™ Blood Collection Set: 21G, 0.75 inch needle, 12 inch tubing with luer adapter; 50 per box, 200 per case	367281* 367286‡
BD Vacutainer One-Use Holder	Case only for 13 mm and 16 mm diameter; 1000/case	364815
BD Vacutainer Plus Serum Tubes	13 x 75 mm 4.0 ml draw with Red BD Hemogard™ closure and paper label; 100/box, 1000/case	367812* 368975‡

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

* USA and Canada.

† Japan.

‡ All other countries.

§ These blood collection accessories represent typical products that can be used with PAXgene Blood DNA Tubes. To find out more about these accessories, including how to order, visit www.bd.com/vacutainer/products/venous.

Notes

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