PreAnalytiX
A QIAGEN / BD Company

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PAXgene® Blood RNA Kit (Handbook) Instructions for Use

Σ 50

Version 3 (V3)

IVD

For In Vitro Diagnostic Use

CE This product fulfills the requirements of the Regulation (EU) 2017/746 on in vitro diagnostic medical devices.

REF 762174

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R1 MAT 1126792

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Contents

Intended Use

For in vitro diagnostic use.

The PAXgene Blood RNA System consists of a blood collection tube (PAXgene Blood RNA Tube, BRT) and nucleic acid purification kit (PAXgene Blood RNA Kit). It is intended for the collection, storage and transport of blood and stabilization of intracellular RNA in a closed tube and subsequent isolation and purification of host RNA from whole blood for RT-PCR used in molecular diagnostic testing.

Performance characteristics for the PAXgene Blood RNA System have only been established with FOS and IL1B gene transcripts. The user is responsible for establishing appropriate PAXgene Blood RNA System performance characteristics for other target transcripts.

Indications for use

The PAXgene Blood RNA Kit is for the purification of intracellular RNA from whole blood collected in the PAXgene Blood RNA Tube (BRT). When the kit is used in conjunction with the PAXgene Blood RNA Tube (BRT), the system provides purified intracellular RNA from whole blood for RT-PCR used in molecular diagnostic testing.

Intended User

The product is intended to be used by professional users, e.g., technicians and physicians who are trained in in vitro diagnostic procedures.

This kit is intended for professional use.

Description and Principle

Introduction

Collection of whole blood is the first step in many molecular assays used to study cellular RNA. However, a major problem in such experiments is the instability of the cellular RNA profile in vitro. Studies at PreAnalytiX have shown that the copy numbers of individual mRNA species in whole blood can change more than 1000-fold during storage or transport at room temperature (Rainen et al., 2002). This is caused both by rapid RNA degradation and by induced expression of certain genes after the blood is drawn. Such changes in the RNA expression profile make reliable studies of gene expression impossible. A method that preserves the RNA expression profile during and after phlebotomy is therefore essential for accurate analysis of gene expression in human whole blood.

Principle and procedure

PreAnalytiX has developed a system that enables the collection, stabilization, storage and transportation of human whole blood specimens, together with a rapid and efficient protocol for isolation of intracellular RNA. The system requires the use of PAXgene Blood RNA Tubes (BRT) for blood collection and RNA stabilization, followed by manual or automated RNA isolation using the PAXgene Blood RNA Kit. Both manual and automated protocols provide substantially equivalent performance with regards to RNA quality and yield. Performance data for the manual protocol (pages 43–51) and the automated protocol (pages 51–53) are included in this handbook.

The PAXgene Blood RNA System enables standardization of the pre-analytical workflow steps from blood specimen collection to cellular RNA isolation according to ISO 20186-1:2019, Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for venous whole blood — Part 1: Isolated cellular RNA.

Sample collection and stabilization

PAXgene Blood RNA Tubes (BRT) contain a proprietary RNA stabilization reagent. This additive protects RNA molecules from degradation by RNases and minimizes ex vivo changes in gene expression. Performance characteristics of the PAXgene Blood RNA System have been established with FOS and IL1B gene transcripts which can be viewed on pages 39–42.

RNA isolation

The PAXgene Blood RNA Kit is for the isolation of total RNA from 2.5 ml human whole blood collected in a PAXgene Blood RNA Tube (BRT). The procedure is simple and can be performed using manual or automated procedures (see Figure 1 or Figure 3, page 9 or 11, respectively). In both protocols, isolation begins with a centrifugation step to pellet nucleic acids in the PAXgene Blood RNA Tube (BRT). The pellet is washed and resuspended, followed by manual or automated RNA isolation. In principle, both protocols follow the same protocol steps with the same kit components.

Manual RNA isolation

In detail, the resuspended pellet is incubated in optimized buffers together with proteinase K (PK) to bring about protein digestion. An additional centrifugation through the PAXgene Shredder spin column (PSC) is carried out to homogenize the cell lysate and remove residual cell debris, and the supernatant of the flow-through fraction is transferred to a fresh microcentrifuge tube (MCT). Ethanol is added to adjust binding conditions, and the lysate is applied to a PAXgene RNA spin column (PRC). During a brief centrifugation, RNA is selectively bound to the PAXgene silica membrane as contaminants pass through. Remaining contaminants are removed in several efficient wash steps. Between the first and second wash steps, the membrane is treated with DNase I (RNFD) to remove trace amounts of bound DNA. After the wash steps, RNA is eluted in elution buffer (BR5) and heat-denatured. Performance characteristics of manual RNA isolation using the PAXgene Blood RNA System can be viewed on page 43.

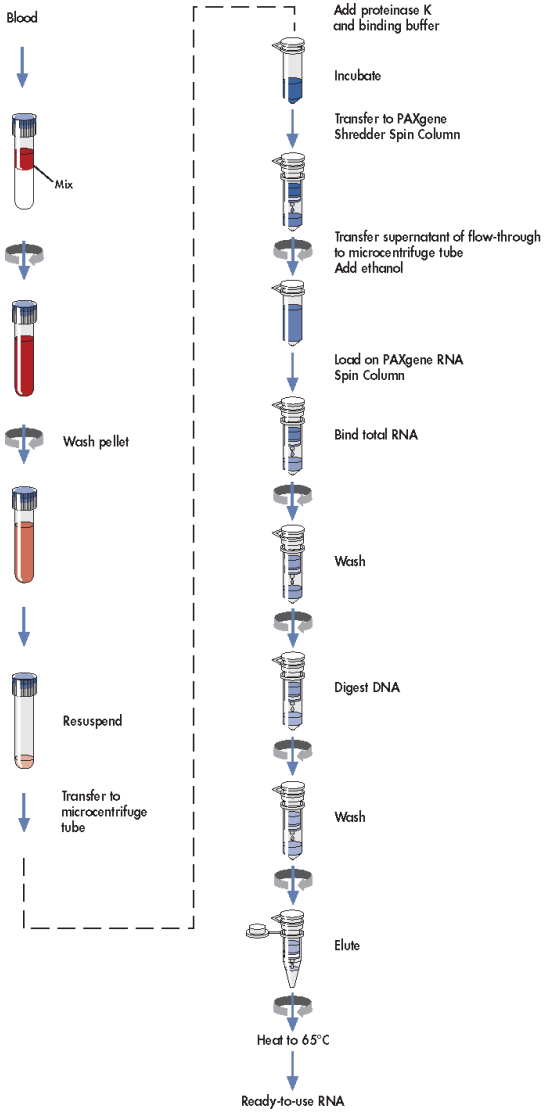


Figure 1: The manual PAXgene Blood RNA procedure.

Automated RNA isolation

Isolation of blood RNA is automated on the QIAGEN QIAcube Connect MDx. The innovative instrument uses advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample prep into laboratory workflow. Sample preparation using the QIAcube Connect MDx follows the same steps as the manual procedure (i.e., lyse, bind, wash and elute), and can be performed using the same PAXgene Blood RNA Kit.

Figure 2: QIAcube Connect MDx.

The QIAGEN QIAcube Connect MDx is not available in all countries. For further details please contact QIAGEN Technical Service.

The automated RNA isolation protocol consists of 2 parts (or protocols), "PAXgene Blood RNA Part A" (from the blood in the PAXgene Blood RNA Tube to elute) and "PAXgene Blood RNA Part B" (after elute to ready-to-use RNA), with a brief manual intervention between the 2 parts (see Figure 3, page 11).

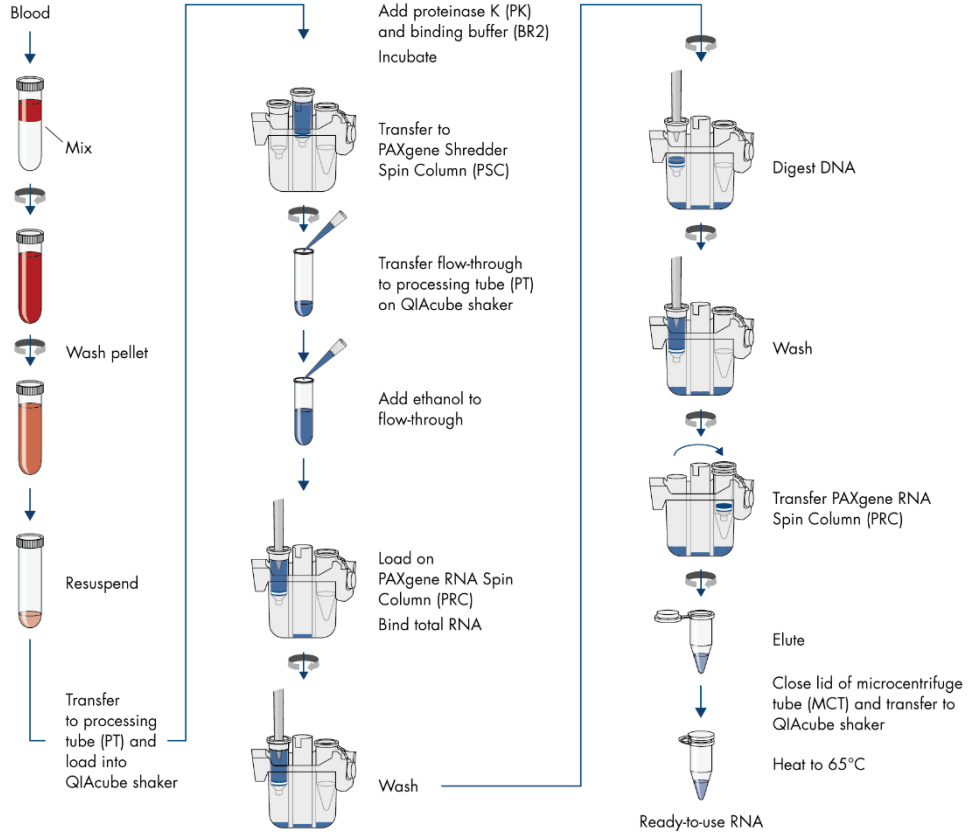


Figure 3: The automated PAXgene Blood RNA procedure.

The centrifuged, washed and resuspended nucleic acid pellet (see "RNA isolation", page 7) is transferred from the PAXgene Blood RNA Tube (BRT) into processing tubes (PTs), which are placed into the thermoshaker unit on the QIAcube Connect MDx' worktable. The operator selects and starts the "PAXgene Blood RNA Part A" protocol from the menu. The QIAcube Connect MDx performs the steps of the protocol through to elution of RNA in elution buffer (BR5). The operator transfers the MCTs containing the purified RNA into the thermoshaker unit of the QIAcube Connect MDx. The operator selects and starts the "PAXgene Blood RNA Part B" protocol from the menu and heat denaturation is performed by the QIAcube Connect MDx. Performance characteristics of automated RNA isolation using the PAXgene Blood RNA System on QIAcube Connect MDx can be viewed on page 51.

Materials Provided

Kit contents

PAXgene Blood RNA Kit Catalog no. Number of collection devices			(50) 762174 50
Component name	**Description**	**Symbol**	**Quantity**
BR1	Resuspension Buffer	RES BUF	20 ml
BR2	Binding Buffer*	BIND BUF	18 ml
BR3	Wash Buffer 1*	WASH BUF 1	45 ml
BR4	Wash Buffer 2 (concentrate)†	WASH BUF 2 CONC	11 ml
BR5	Elution Buffer	ELU BUF	6 ml
RNFW	RNase-Free Water (bottle)	PEL WASH	2 x 125 ml
PK	Proteinase K (green lid)	PROTK	2 x 1.4 ml
PRC	PAXgene RNA Spin Columns (red)‡	PAXgene RNA COL	5 x 10
PT	Processing Tubes (2 ml)§	PROC TUBE	6 x 50
Hemogard™	Secondary BD Hemogard Closures	SEC CLOS	50
MCT	Microcentrifuge Tubes (1.5 ml)§	MIC TUBE	3 x 50, 1 x 10
RNFD	DNase I, RNase-free (lyophilized)	DNA REM	1500 Kunitz units¶
RDD	DNA Digestion Buffer (white lid)	DNA DIG BUF	2 x 2 ml
DRB	DNase Resuspension Buffer (tube, lilac lid)	DNase RES BUF	2 ml
PSC	PAXgene Shredder Spin Columns (lilac)‡	PAXgene SHRED COL	5 x 10
Handbook	PAXgene Blood RNA Kit Handbook (Version 3)		1

* Not compatible with disinfecting reagents containing bleach. Contains a guanidine salt. See page 17 for Safety information.

† Wash Buffer 2 (BR4) is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100% v/v, purity grade p.a.) as indicated on the bottle to obtain a working solution.

‡ Each column is packed in a blister that is intended for single use only. Please see safety information for disposal instructions.

§ Tubes are available in plastic bags, and each tube is intended for single use only. Please see safety information for disposal instructions.

¶ Kunitz units are the commonly used units for measuring DNase I, defined as the amount of DNase I that causes an increase in A_{260} of 0.001 per minute per milliliter at 25°C, pH 5.0, with highly polymerized DNA as the substrate (*Kunitz, M. (1950) J. Gen. Physiol. 33, 349 and 363*).

Components of the kit

Component name	Description	Active Ingredient	Concentration
BR1	Resuspension Buffer	None	-
BR2	Binding Buffer	Guanidine thiocyanate	≥ 30 to < 50% w/w
BR3	Wash Buffer 1	Guanidine thiocyanate Ethanol	≥ 10 to < 20% w/w ≥ 3 to < 10% w/w
BR4	Wash Buffer 2 (concentrate)	None	-
BR5	Elution Buffer	None	-
RNFW	RNase-Free Water (bottle)	None	-
PK	Proteinase K (green lid)	Proteinase K	≥ 1 to < 3% w/w
RNFD	DNase I, RNase-free (lyophilized)	DNase	≥ 90 to ≤ 100% w/w
RDD	DNA Digestion Buffer (white lid)	None	-
DRB	DNase Resuspension Buffer (tube, lilac lid)	None	-

Materials Required but Not Provided

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.

For all protocols

- PAXgene Blood RNA Tubes (BRT, PreAnalytiX; cat. no. 762165)
- Ethanol (96–100% v/v, purity grade p.a.)
- Pipets* (10 µl – 4 ml)
- Sterile, aerosol-barrier, RNase-free pipet tips†
- Graduated cylinder‡
- Centrifuge* capable of attaining 3000–5000 x *g* and equipped with a swing-bucket rotor to hold PAXgene Blood RNA Tubes (BRT)
- Vortex mixer*
- Crushed ice
- Permanent pen for labeling

For the manual protocol

- Variable-speed microcentrifuge* capable of attaining a range of at least 1000–8000 x *g*, though lower and higher *g*-forces are applicable (see the protocol steps for details), and equipped with a rotor for 2 ml MCTs

* Make sure that the devices and instruments have been checked, maintained and calibrated regularly according to the manufacturer's recommendations.

† Make sure that you are familiar with the guidelines on handling RNA (Appendix A, page 78).

‡ For the addition of ethanol to Buffer BR4 concentrate.

- Shaker–incubator* capable of incubating at 55°C and 65°C and shaking at ≥400 rpm, not exceeding 1400 rpm (e.g., Eppendorf® Thermomixer Compact or equivalent)

For the automated protocol

- Scissors
- QIAcube Connect MDx* (QIAGEN, cat. no. 9003070)

QIAcube Connect MDx consumables:

- Filter-Tips, 1000 µl (1024) (QIAGEN, cat. no. 990352)†
- Reagent Bottles, 30 ml (6) (QIAGEN, cat. no. 990393)†
- Rotor Adapters (10 x 24) (QIAGEN, cat. no. 990394)†

QIAcube Connect MDx accessories:

- Rotor Adapter Holder (QIAGEN, cat. no. 990392)†

QIAcube Connect MDx service bundles:

- QIAcube Connect MDx System FUL-2 (QIAGEN, cat. no. 9003071)
- QIAcube Connect MDx System FUL-3 (QIAGEN, cat. no. 9003072)
- QIAcube Connect MDx System PRV-1 (QIAGEN, cat. no. 9003073)
- QIAcube Connect MDx Device PRV-1 (QIAGEN, cat. no. 9003074)
- QIAcube Connect MDx System PRM-1 (QIAGEN, cat. no. 9003075)

* Make sure that the device and instrument has been checked, maintained and calibrated regularly according to the manufacturer's recommendations.

† Also included in the Starter Pack, QIAcube (QIAGEN, cat. no. 990395).

Warnings and Precautions

For customers in the European Union, please be aware that you are required to report any serious incident that has occurred in relation to the device to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

For customers outside European Union, please be aware that you may be required to consult your local regulations for reporting serious incidents that have occurred in relation to the device to the manufacturer and/or its authorized representative and the regulatory authority in which the user and/or the patient is established.

Safety information

When working with chemicals and biohazardous materials, 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 QIAGEN kit and kit component.

- All chemicals and biological materials are potentially hazardous. Blood specimens and samples are potentially infectious and must be treated as biohazardous materials.
- Discard biohazardous waste and kit waste according to your local safety procedures.

Emergency information

CHEMTREC
Outside USA & Canada +1 703-527-3887

Precautions

When working with blood practice universal precautions to avoid risk of potential exposure to bloodborne pathogens (e.g. HIV, hepatitis B and other bloodborne viruses). Use gloves, gowns, eye protection, other personal protective equipment and engineering controls to protect from blood exposure. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in a convenient and compact PDF format at **www.preanalytix.com** where you can find, view and print the SDSs for this kit.

CAUTION

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

Binding buffer (BR2) and wash buffer 1 (BR3) contain guanidine thiocyanate, which can form highly reactive compounds when combined with bleach. If binding buffer (BR2) or wash buffer 1 (BR3) are spilt, clean with suitable laboratory detergent and water. If liquid containing potentially infectious agents is spilt, clean the affected area first with laboratory detergent and water and then with 1% (v/v) sodium hypochlorite (bleach).

The RNA stabilizing solution and blood mixture from the PAXgene Blood RNA Tube (BRT) can be disinfected using 1 volume of commercial bleach solution (5% sodium hypochlorite) per 9 volumes of the RNA stabilizing solution and blood mixture.

Sample preparation waste, such as supernatants from centrifugation steps in the RNA isolation procedure, is to be considered potentially infectious. Use biohazard

containers to dispose biological materials. Disposal must be made according to local regulations and procedures of your facility.

Specific components of the PAXgene Blood RNA Kit are intended for single use only. See the Kit contents on page 13 for information on individual components.

The following hazard and precautionary statements apply to components of the PAXgene Blood RNA Kit. See the *PAXgene Blood RNA Tube Handbook* for safety information about PAXgene Blood RNA Tubes (BRT).

Buffer BR2

Contains: guanidine thiocyanate. Danger! Harmful if swallowed. May be harmful in contact with skin or if inhaled. Causes serious eye damage. Harmful to aquatic life with long lasting effects. Contact with acids liberates very toxic gas. Wear protective gloves/protective clothing/eye protection/face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor/ physician.

Buffer BR3

Contains: ethanol; guanidine thiocyanate. Danger! Flammable liquid and vapor. Causes serious eye damage. Contact with acids liberates very toxic gas. Keep away from heat/sparks/open flames/hot surfaces. No smoking. Wear protective gloves/ protective clothing/eye protection/face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor/physician.

DNase I

Contains: DNase. Danger! May cause an allergic skin reaction. May cause allergy or asthma symptoms or breathing difficulties if inhaled. Avoid breathing dust/fume/gas/mist/vapors/spray. Wear protective gloves/ protective clothing/ eye protection/ face protection. Wear respiratory protection. IF exposed or concerned: Call a POISON CENTER or doctor/ physician. Remove victim to fresh air and keep at rest in a position comfortable for breathing.

Proteinase K

Contains: proteinase K. Danger! Causes mild skin irritation. May cause allergy or asthma symptoms or breathing difficulties if inhaled. Avoid breathing dust/fume/gas/mist/vapors/spray. Wear protective gloves/protective clothing/ eye protection/ face protection. Wear respiratory protection. IF exposed or concerned: Call a poison center or doctor/physician. Remove victim to fresh air and keep at rest in a position comfortable for breathing.

Reagent Storage and Handling

PAXgene RNA spin columns (PRC), PAXgene Shredder spin columns (PSC), proteinase K (PK) and buffers (BR1, BR2, BR3, BR4 and BR5) should be stored dry at the temperature indicated on the kit label.

The RNase-Free DNase Set, which contains DNase I (RNFD), DNA digestion buffer (RDD) and DNase resuspension buffer (DRB), is shipped at ambient temperature. Store all components of the RNase-Free DNase Set immediately upon receipt at the temperature indicated on the label. When stored properly, the kit is stable until the expiration date on the kit box.

Attention should be paid to expiration dates and storage conditions printed on the box and labels of all components. Do not use expired or incorrectly stored components.

In-use stability

After first use of the kit, reagents are stable in the original bottles at temperatures and until the expiration date indicated on the kit box label.

Reagents filled into the reagent bottles of the QIAcube Connect MDx are stable for 3 months of storage at room temperature (15–25°C).

Reconstituted DNase I (RNFD) is stable at 2–8°C for 6 weeks in the original glass vial (stock solution).

Single-use aliquots of the stock solution in 1.5 ml MCTs (supplied with the kit) are stable for 9 months of storage at –20°C. After thawing, the single-use aliquots are stable for 6 weeks of storage at 2–8°C.

Specimen Collection, Storage and Handling

The PAXgene Blood RNA Kit is for use with blood collected in the PAXgene Blood RNA Tubes. Blood must be collected in PAXgene Blood RNA Tubes (BRT) according to the instructions in the PAXgene Blood RNA Tube Handbook. If necessary, see Appendix C (page 77) for recommendations on handling PAXgene Blood RNA Tubes (BRT). All samples should be treated as potentially hazardous. Performance characteristics of the PAXgene Blood RNA System have been established with FOS and IL1B gene transcripts which can be viewed on pages 39–42.

Protocol: Manual Isolation of Total RNA from Human Whole Blood Collected into PAXgene Blood RNA Tubes

Important points before starting

- Make sure that the kit box is intact and undamaged and that buffers have not leaked. Do not use a kit that has been damaged.
- When using a pipet, ensure that it is set to the correct volume and that liquid is carefully and completely aspirated and dispensed.
- To avoid transferring samples to the wrong tube or spin column, ensure that all tubes and spin columns are properly labeled using a permanent pen. Label the lid and the body of each tube (PT, MCT). For spin columns, label the body of its PT. Close each tube or spin column after liquid is transferred to it.
- Spillages of samples and buffers during the procedure may reduce the yield and purity of RNA.
- Unless otherwise indicated, all steps of this protocol, including centrifugation steps, should be carried out at room temperature (15–25°C).

Because of the sensitivity of nucleic acid amplification technologies, the following precautions are necessary when handling samples to avoid cross-contamination:

- Carefully pipet the sample into the spin column (PSC, PRC) without moistening the rim of the column.
- Always change pipet tips between liquid transfers. Use aerosol-barrier pipet tips.
- Avoid touching the spin column (PSC, PRC) membrane with the pipet tip.
- After vortexing or heating a MCT, briefly centrifuge it to remove drops from the inside of the lid.

- Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.
- Close the spin column (PSC, PRC) before placing it in the microcentrifuge. Centrifuge as described in the procedure.
- Open only one spin column (PSC, PRC) at a time and take care to avoid generating aerosols.
- For efficient parallel processing of multiple samples, fill a rack with PTs to which the spin columns (PSC, PRC) can be transferred after centrifugation. Discard the used PTs containing flow-through, and place the spin columns (PSC, PRC) into new PTs before transferring back into the microcentrifuge.

Things to do before starting

- Blood must be collected in PAXgene Blood RNA Tubes (BRT) according to the instructions in the *PAXgene Blood RNA Tube Handbook*. If necessary, see Appendix C (page 77) for recommendations on handling PAXgene Blood RNA Tubes (BRT).
- Ensure that the PAXgene Blood RNA Tubes (BRT) are incubated for at least 2 h at room temperature after blood collection to ensure complete lysis of blood cells and precipitation of RNA. Incubation of the PAXgene Blood RNA Tube (BRT) overnight may increase yields. If the initial blood incubation at room temperature for 2 h was not done before storage at 2–8°C, −20°C or −70°C, then first equilibrate the PAXgene Blood RNA Tube (BRT) to room temperature and then incubate it at this temperature for 2 h before starting the procedure.
- Read the safety information on page 17.
- Read the guidelines on handling RNA (Appendix A, page 74).
- Ensure that instruments, such as pipets and the shaker–incubator, have been checked and calibrated regularly according to the manufacturer's recommendations.

- A shaker–incubator is required in steps 5 and 20. Set the temperature of the shaker–incubator to 55°C.
- Binding buffer (BR2) may form a precipitate upon storage. If necessary, warm to 37°C to dissolve.
- Wash buffer 2 (BR4) is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100% v/v, purity grade p.a.) as indicated on the bottle to obtain a working solution.
- If using the RNase-Free DNase Set for the first time, prepare DNase I stock solution. Dissolve the solid DNase I (RNFD; 1500 Kunitz units)* in 550 µl of the DNase resuspension buffer (DRB) provided with the set. Take care that no DNase I (RNFD) is lost when opening the vial. Do not vortex the reconstituted DNase I (RNFD). DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the vial.
- Reconstituted DNase I (RNFD) can be stored at 2–8°C in the original glass vial (stock solution) or at –20°C after removing the stock solution from the glass vial and dividing it into single-use aliquots (use the 1.5 ml MCT supplied with the kit; there are enough for 5 aliquots). Thawed aliquots can be stored at 2–8°C. Do not refreeze the aliquots after thawing.
- When reconstituting and aliquoting DNase I (RNFD), ensure that you follow the guidelines for handling RNA (Appendix A, page 74).

Procedure

1. Centrifuge the PAXgene Blood RNA Tube (BRT) for 10 min at 3000–5000 x *g* using a swing-bucket rotor.

* Kunitz units are the commonly used units for measuring DNase I, defined as the amount of DNase I that causes an increase in A_{260} of 0.001 per minute per milliliter at 25°C, pH 5.0, with highly polymerized DNA as the substrate (Kunitz, M. (1950) J. Gen. Physiol. **33**, 349 and 363).

Ensure that the blood sample has been incubated in the PAXgene Blood RNA Tube (BRT) for a minimum of 2 h at room temperature (15–25°C) to achieve complete lysis of blood cells and precipitation of RNA.

The rotor must contain tube adapters for round-bottom tubes. If other types of tube adapter are used, the tubes may break during centrifugation.

2. Remove the supernatant by decanting or pipetting. Add 4 ml RNase-Free Water (RNFW) to the pellet and close the tube using a fresh secondary BD Hemogard closure (supplied with the kit).

   If the supernatant is decanted, take care not to disturb the pellet and dry the rim of the tube with a clean paper towel.

3. Vortex until the pellet is visibly dissolved and centrifuge for 10 min at 3000–5000 x *g* using a swing-bucket rotor. Remove and discard the entire supernatant.

   Small debris remaining in the supernatant after vortexing but before centrifugation will not affect the procedure.

   Incomplete removal of the supernatant will inhibit lysis and dilute the lysate, and therefore affect the conditions for binding RNA to the PAXgene membrane.

4. Add 350 µl resuspension buffer (BR1) and vortex until the pellet is visibly dissolved.

5. Pipet the sample into a 1.5 ml MCT. Add 300 µl binding buffer (BR2) and 40 µl proteinase K (PK). Mix by vortexing for 5 s and incubate for 10 min at 55°C using a shaker–incubator at 400–1400 rpm. After incubation, set the temperature of the shaker–incubator to 65°C (for step 20).

   Do not mix binding buffer (BR2) and proteinase K (PK) together before adding them to the sample.

6. Pipet the lysate directly into a PSC (lilac) placed in a 2 ml PT and centrifuge for 3 min at maximum speed (but not to exceed 20,000 x *g*).

Carefully pipet the lysate into the spin column (PSC) and visually check that the lysate is completely transferred to the spin column (PSC).

To prevent damage to columns (PSC) and tubes (PT), do not exceed 20,000 x *g*.

Some samples may flow through the PSC without centrifugation. This is due to low viscosity of some samples and should not be taken as an indication of product failure.

7. Carefully transfer the entire supernatant of the flow-through fraction to a fresh 1.5 ml MCT without disturbing the pellet in the PT.
8. Add 350 µl ethanol (96–100% v/v, purity grade p.a.). Mix by vortexing and centrifuge briefly (1–2 s at 500–1000 x *g*) to remove drops from the inside of the tube lid.

The length of the centrifugation must not exceed 1–2 s, as this may result in pelleting of nucleic acids and reduced yields of total RNA.

9. Pipet 700 µl sample into the PRC (red) placed in a 2 ml PT and centrifuge for 1 min at 8000–20,000 x *g*. Place the spin column (PRC) in a new 2 ml PT and discard the old PT containing flow-through.
10. Pipet the remaining sample into the PRC and centrifuge for 1 min at 8000–20,000 x *g*. Place the spin column (PRC) in a new 2 ml PT and discard the old PT containing flow-through.

Carefully pipet the sample into the spin column (PRC) and visually check that the sample is completely transferred to the spin column (PRC).

11. Pipet 350 µl wash buffer 1 (BR3) into the PRC. Centrifuge for 1 min at 8000–20,000 x *g*. Place the spin column (PRC) in a new 2 ml PT and discard the old PT containing flow-through.
12. Add 10 µl DNase I (RNFD) stock solution to 70 µl DNA digestion buffer (RDD) in a 1.5 ml MCT. Mix by gently flicking the tube and centrifuge briefly to collect residual liquid from the sides of the tube.

    If processing, for example, 10 samples, add 100 µl DNase I (RNFD) stock solution to 700 µl DNA digestion buffer (RDD). Use the 1.5 ml MCTs supplied with the kit.

DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently flicking the tube. Do not vortex.

13. Pipet the DNase I (RNFD) incubation mix (80 µl) directly onto the PRC membrane and place on the benchtop (20–30°C) for 15 min.

Ensure that the DNase I (RNFD) incubation mix is placed directly onto the membrane. DNase digestion will be incomplete if part of the mix is applied to and remains on the walls or the O-ring of the spin column (PRC).

14. Pipet 350 µl wash buffer 1 (BR3) into the PRC and centrifuge for 1 min at 8000–20,000 x *g*. Place the spin column (PRC) in a new 2 ml PT and discard the old PT containing flow-through.
15. Pipet 500 µl wash buffer 2 (BR4) into the PRC and centrifuge for 1 min at 8000–20,000 x *g*. Place the spin column (PRC) in a new 2 ml PT and discard the old PT containing flow-through.

Wash buffer 2 (BR4) is supplied as a concentrate. Ensure that ethanol is added to wash buffer 2 (BR4) before use (see "Things to do before starting", page 24).

16. Add another 500 µl wash buffer 2 (BR4) to the PRC. Centrifuge for 3 min at 8000–20,000 x *g*.
17. Discard the PT containing the flow-through and place the PRC in a new 2 ml PT. Centrifuge for 1 min at 8000–20,000 x *g*.
18. Discard the PT containing the flow-through. Place the PRC in a 1.5 ml MCT and pipet 40 µl elution buffer (BR5) directly onto the PRC membrane. Centrifuge for 1 min at 8000–20,000 x *g* to elute the RNA.

    It is important to wet the entire membrane with elution buffer (BR5) to achieve maximum elution efficiency.

19. Repeat the elution step (step 18) as described, using 40 µl elution buffer (BR5) and the same MCT.

20. Incubate the eluate for 5 min at 65°C in the shaker–incubator (from step 5) without shaking. After incubation, chill immediately on ice.

    This incubation of samples at 65°C denatures the RNA for downstream applications. Even if the downstream application includes a heat denaturation step, do not omit this step. Sufficient RNA denaturation at this point is essential for maximum efficiency in downstream applications.

    Do not exceed the incubation time or temperature.

21. If the RNA samples will not be used immediately, store at −20°C or −70°C.

    Since the RNA remains denatured after repeated freezing and thawing, it is not necessary to repeat the incubation at 65°C. If using the RNA samples in a diagnostic assay, follow the instructions supplied by the manufacturer.

    For accurate quantification of RNA by absorbance at 260 nm, we recommend diluting samples with 10 mM Tris-HCl, pH 7.5.* Diluting the sample in RNase-Free Water may lead to inaccurately low values.

    Zero the spectrophotometer using a blank consisting of the same proportion elution buffer (BR5) and Tris-HCl buffer as in the samples to be measured. Elution buffer (BR5) has high absorbance at 220 nm, which can lead to high background absorbance levels if the spectrophotometer is not properly zeroed.

    For quantification in Tris HCl buffer, use the relationship $A_{260} = 1 \Rightarrow 44$ µg/ml. See Appendix B, page 75.

22. Reclose all bottles containing buffers and RNase-free water, vials and tubes containing enzymes and enzyme buffers, and bags containing plastic materials from the kit used for the protocol. Store the remaining contents of the kit as described in the section “Reagent Storage and Handling” (page 21) and “In-use stability” (page 21) until further use.

* 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.

Protocol: Automated Isolation of Total RNA from Human Whole Blood Collected into PAXgene Blood RNA Tubes (BRT)

Important points before starting

- Make sure that the kit box is intact and undamaged and that buffers have not leaked. Do not use a kit that has been damaged.
- When using a pipet, ensure that it is set to the correct volume and that liquid is carefully and completely aspirated and dispensed.
- To avoid transferring samples to the wrong tubes and plastic consumables, ensure that all PT, MCTs and rotor adapters are properly labeled using a permanent pen. Label the lid and the body of each MCT, the body of each PT and the outer wall of each rotor adapter.
- Spillages of samples and buffers during the procedure may reduce the yield and purity of RNA.
- Unless otherwise indicated, all steps of this protocol, including centrifugation steps, should be carried out at room temperature (15–25°C).

Because of the sensitivity of nucleic acid amplification technologies, the following precautions are necessary when handling samples to avoid cross-contamination:

- Carefully pipet the sample into the PT, into the bottom of the tube without moistening the rim of the tube.
- Always change pipet tips between liquid transfers. Use aerosol-barrier pipet tips.
- Avoid touching the spin column (PSC, PRC) membrane with the pipet tip.
- After vortexing or heating a MCT, briefly centrifuge it to remove drops from the inside of the lid.

- Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

Things to do before starting

- Blood must be collected in PAXgene Blood RNA Tubes (BRT) according to the instructions in the *PAXgene Blood RNA Tube Handbook*. If necessary, see Appendix C (page 77) for recommendations on handling PAXgene Blood RNA Tubes (BRT).
- Ensure that the PAXgene Blood RNA Tubes (BRT) are incubated for at least 2 h at room temperature after blood collection to ensure complete lysis of blood cells and precipitation of RNA. Incubation of the PAXgene Blood RNA Tube (BRT) overnight may increase yields. If the PAXgene Blood RNA Tube (BRT) was stored at 2–8°C, −20°C or −70°C after blood collection, first equilibrate it to room temperature and then store it at room temperature for 2 h before starting the procedure.
- Read the safety information on page 17.
- Read "Important Notes", page 55.
- Read the guidelines on handling RNA (Appendix A, page 74).
- Read the appropriate QIAcube Connect MDx User Manual and any additional information supplied with the instrument, paying careful attention to the safety information.
- Ensure that devices and instruments, such as pipets and the QIAcube Connect MDx, have been checked and calibrated regularly according to the manufacturer's recommendations.
- Binding buffer (BR2) may form a precipitate upon storage. If necessary, warm to 37°C to dissolve.
- Wash buffer 2 (BR4) is supplied as a concentrate. Before using for the first time, add the appropriate volume of ethanol (96–100% v/v, purity grade p.a.) as indicated on the bottle to obtain a working solution.

- If using the RNase-Free DNase Set for the first time, prepare DNase I stock solution. Dissolve the solid DNase I (RNFD; 1500 Kunitz units)* in 550 µl of the DNase resuspension buffer (DRB) provided with the set. Take care that no DNase I (RNFD) is lost when opening the vial. Do not vortex the reconstituted DNase I (RNFD). DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the vial.
- Reconstituted DNase I (RNFD) can be stored at 2–8°C in the original glass vial (stock solution) or at –20°C after removing the stock solution from the glass vial and dividing it into single-use aliquots (use the 1.5 ml MCT supplied with the kit; there are enough for 5 aliquots). Thawed aliquots can be stored at 2–8°C. Do not refreeze the aliquots after thawing.
- When reconstituting and aliquoting DNase I (RNFD), ensure that you follow the guidelines for handling RNA (Appendix A, page 74).
- Install the correct shaker adapter (included with the QIAcube Connect MDx; use the adapter for 2 ml safe-lock tubes, marked with a "2"), and place the shaker rack on top of the adapter.
- Check the waste drawer and empty it if necessary.
- Install any related protocols if not already done for previous runs. The QIAcube Connect MDx requires all protocols found in the related zip file to be downloaded. See "Installing protocols on the QIAcube Connect MDx", page 57.

Procedure

1. Close the QIAcube Connect MDx hood, and switch on the instrument with the power switch (see Figure 15, page 56).

   A beeper sounds and the startup screen appears. The instrument automatically performs initialization tests.

* Kunitz units are the commonly used units for measuring DNase I, defined as the amount of DNase I that causes an increase in A_{260} of 0.001 per minute per milliliter at 25°C, pH 5.0, with highly polymerized DNA as the substrate (Kunitz, M. (1950) J. Gen. Physiol. **33**, 349 and 363).

2. Open the QIAcube Connect MDx hood, and load the necessary reagents and plasticware into the instrument. See “Loading the QIAcube Connect MDx”, page 58.

   To save time, loading can be performed during one or both of the following 10 min centrifugation steps (steps 3 and 5).

3. Centrifuge the PAXgene Blood RNA Tube (BRT) for 10 min at 3000–5000 x *g* using a swing-bucket rotor.

   Ensure that the blood sample has been incubated in the PAXgene Blood RNA Tube (BRT) for a minimum of 2 h at room temperature (15–25°C), to achieve complete lysis of blood cells and precipitation of RNA.

   The rotor must contain tube adapters for round-bottom tubes. If other types of tube adapter are used, the tubes may break during centrifugation.

4. Remove the supernatant by decanting or pipetting. If the supernatant is decanted, take care not to disturb the pellet and dry the rim of the tube with a clean paper towel. Add 4 ml RNase-Free Water (RNFW) to the pellet and close the tube using a fresh secondary BD Hemogard closure (supplied with the kit).

5. Vortex until the pellet is visibly dissolved and centrifuge for 10 min at 3000–5000 x *g* using a swing-bucket rotor. Remove and discard the entire supernatant.

   Small debris remaining in the supernatant after vortexing but before centrifugation will not affect the procedure.

   Incomplete removal of the supernatant will inhibit lysis and dilute the lysate, and therefore affect the conditions for binding RNA to the PAXgene membrane.

6. Add 350 µl resuspension buffer (BR1) and vortex until the pellet is visibly dissolved.

7. Pipet the sample into a 2 ml PT.

Use the 2 ml PTs included in the PAXgene Blood RNA Kit.

8. Load the open PTs containing sample into the QIAcube Connect MDx shaker (see Figure 18, page 60). The sample positions are numbered for ease of loading. Insert shaker rack plugs (included with the QIAcube Connect MDx) into the slots at the edge of the shaker rack next to each PT. This enables detection of samples during the load check.

   Make sure that the correct shaker adapter (Shaker Adapter, 2 ml, safe-lock tubes, marked with a "2", included with the QIAcube Connect MDx) is installed.

   If processing fewer than 12 samples, make sure to load the shaker rack as shown in Figure 22, page 64. One (1) or 11 samples cannot be processed. The position numbers in the shaker rack correspond to the position numbers in the centrifuge.

9. Close the QIAcube Connect MDx hood (see Figure 15, page 56).

10. Select the "PAXgene Blood RNA Part A" protocol and start the protocol.

    Follow the instructions given on the touchscreen of the QIAcube Connect MDx.

    Make sure that both program parts (part A and part B) are installed on the QIAcube Connect MDx (see "Installing protocols on the QIAcube Connect MDx", page 57).

    The instrument will perform load checks for samples, tips, rotor adapters and reagent bottles.

11. After the "PAXgene Blood RNA Part A" protocol is finished, open the QIAcube Connect MDx hood (see Figure 15, page 56). Remove and discard the PRC from the rotor adapters and the empty PTs from the shaker.

    During the run, spin columns are transferred from the rotor adapter position 1 (lid position L1) to rotor adapter position 3 (lid position L2) by the instrument (see Figure 20, page 62).

12. Close the lids of all 1.5 ml MCTs containing the purified RNA in the rotor adapters (position 3, lid position L3, see Figure 20, page 62). Transfer the 1.5 ml MCTs onto the QIAcube Connect MDx shaker adapter (see Figure 18, page 60).
13. Close the QIAcube Connect MDx hood (see Figure 15, page 56).
14. Select the "PAXgene Blood RNA Part B" protocol and start the protocol.

    Follow the instructions given on the QIAcube Connect MDx touchscreen.

    This program incubates the samples at 65°C and denatures the RNA for downstream applications. Even if the downstream application includes a heat denaturation step, do not omit this step. Sufficient RNA denaturation at this point is essential for maximum efficiency in downstream applications.
15. After the "PAXgene Blood RNA Part B" program is finished, open the QIAcube Connect MDx hood (see Figure 15, page 56). Immediately place the MCTs containing the purified RNA on ice.

WARNING: Hot surface. The shaker can reach temperatures of up to 70°C (158°F). Avoid touching it when it is hot.

Do not let the purified RNA remain in the QIAcube Connect MDx. Since the samples are not cooled, the purified RNA can be degraded. Unattended overnight sample preparation runs are therefore not recommended.

16. If the RNA samples will not be used immediately, store at −20°C or −70°C.

    Since the RNA remains denatured after repeated freezing and thawing, it is not necessary to repeat the heat incubation protocol ("PAXgene Blood RNA Part B"). If using the RNA samples in a diagnostic assay, follow the instructions supplied by the manufacturer.

For accurate quantification of RNA by absorbance at 260 nm, we recommend diluting samples in 10 mM Tris-HCl, pH 7.5.* Diluting the sample in RNase-Free Water may lead to inaccurately low values.

Zero the spectrophotometer using a blank consisting of the same proportion elution buffer (BR5) and Tris-HCl buffer as in the samples to be measured. Elution buffer (BR5) has high absorbance at 220 nm, which can lead to high background absorbance levels if the spectrophotometer is not properly zeroed.

For quantification in Tris-HCl buffer, use the relationship $A_{260} = 1 \Rightarrow 44$ µg/ml. See Appendix B, page 75.

17. Remove the reagent bottle rack from QIAcube Connect MDx worktable (see Figure 18, page 60), and close all reagent bottles with the appropriately labeled lids. Reclose all bottles containing buffers and RNase-free water, vials and tubes containing enzymes and enzyme buffers, and bags containing plastic materials from the kit used for the protocol. Store the remaining contents of the kit and the reagent bottles as described in the section "Reagent Storage and Handling" (page 21) and "In-use stability" (page 21) until further use.

    Remove and discard remaining reagents in the PTs in the QIAcube Connect MDx MCT slots. Remove and discard rotor adapters from the centrifuge. Empty the QIAcube Connect MDx waste drawer (see Figure 15, page 56). Close the instrument hood and switch off the instrument with the power switch.

* 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.

Product Use Limitations

The PAXgene Blood RNA Kit is intended for isolation of intracellular RNA from human whole blood (4.8×10^6 – 1.1×10^7 leukocytes/ml) for in vitro diagnostics applications. It is not for the isolation of genomic DNA or viral nucleic acids from human whole blood. Due to the limited number of transcripts validated for stabilization specifications (FOS and IL1B gene transcripts), the performance characteristics have not been established for all transcripts. Users should review the manufacturer's data and their own data to determine whether validation is necessary for other transcripts. The components of the kit are only intended to be used in the manual and automated protocol described in this instructions for use.

See the *PAXgene Blood RNA Tube Handbook* for information about the use of PAXgene Blood RNA Tubes (BRT).

Quality Control

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

Performance Characteristics

Sample collection and stabilization

PAXgene Blood RNA Tubes (BRT) contain a proprietary RNA stabilization reagent. This additive protects RNA molecules from degradation by RNases and minimizes ex vivo changes in gene expression. PAXgene Blood RNA Tubes (BRT) are intended for the collection of human whole blood and stabilization of cellular RNA for up to 3 days at 18–25°C (Figure 4 and Figure 5, pages 39 and 40, respectively) or up to 5 days at 2–8°C (Figure 6 and Figure 7, pages 41 and 42). In addition, stabilized blood can be stored frozen. Currently available data shows stabilization of cellular RNA for at least 11 years at −20°C or −70°C*. For more information from ongoing studies evaluating stability for longer time periods, please visit **www.preanalytix.com** or contact QIAGEN Technical Services.

The actual duration of RNA stabilization may vary depending upon the species of cellular RNA and the downstream application used. Due to the limited number of transcripts validated for stabilization specifications (FOS and IL1B gene transcripts), the performance characteristics have not been established for all transcripts. Users should review the manufacturer's data and their own data to determine whether validation is necessary for other transcripts.

* A long-term study of blood storage in PAXgene Blood RNA Tubes is ongoing.

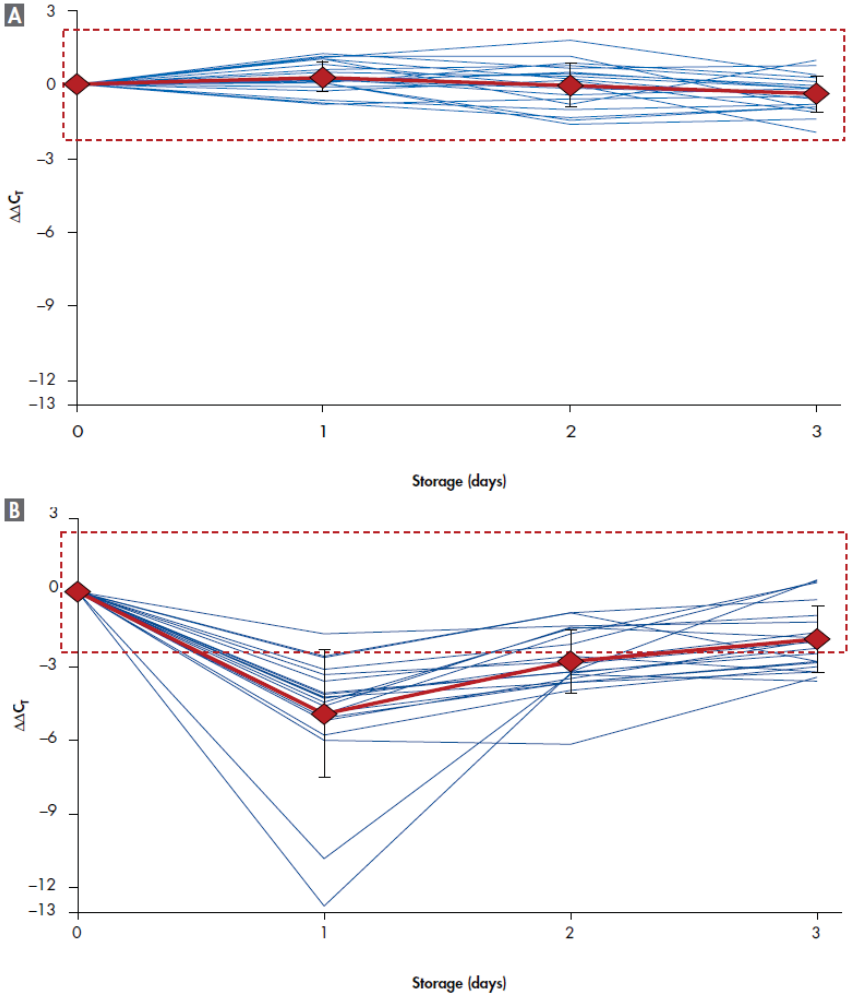


Figure 4: RNA stability in blood samples at 18–25°C: FOS. Blood was drawn from 10 apparently healthy donors, with duplicate samples and stored at 18–25°C for the indicated number of days, followed by total RNA isolation. [A] Blood was collected and stored in PAXgene Blood RNA Tubes (BRT), and total RNA was purified using the PAXgene Blood RNA Kit. [B] Blood was collected and stored in standard blood collection tubes with EDTA as an anticoagulant, and total RNA was purified using a standard organic-isolation method with silica-membrane-based RNA cleanup. Relative transcript levels of FOS were determined by real-time, duplex RT-PCR, using 18S rRNA as an internal standard. The values for all samples are plotted, with means and standard deviations of all samples shown. The dashed lines indicate the ±3x total precision of the assay (2.34 C_T).

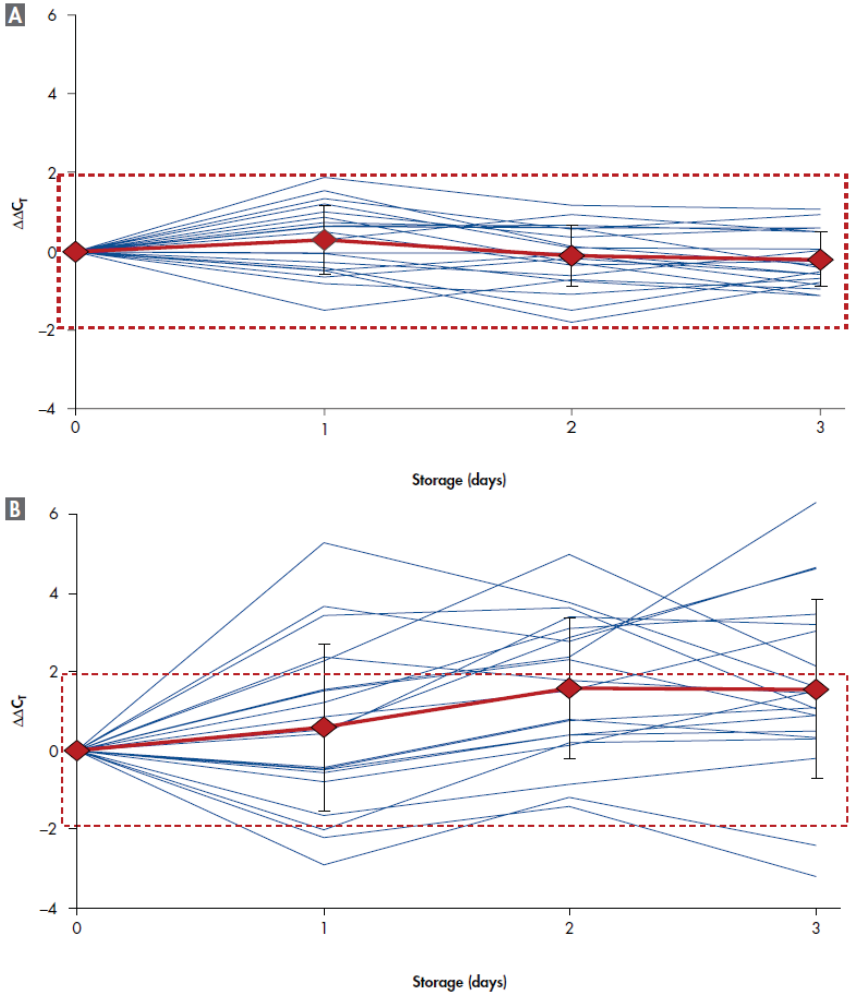


Figure 5: RNA stability in blood samples at 18–25°C: IL1B. Blood was drawn and total RNA purified, after storage at 18–25°C, as described in Figure 4. Relative transcript levels of IL1B were determined by real-time, duplex RT-PCR, using 18S rRNA as an internal standard. The values for all samples are plotted, with means and standard deviations of all samples shown. The dashed lines indicate the ±3x total precision of the assay (1.93 C_T).

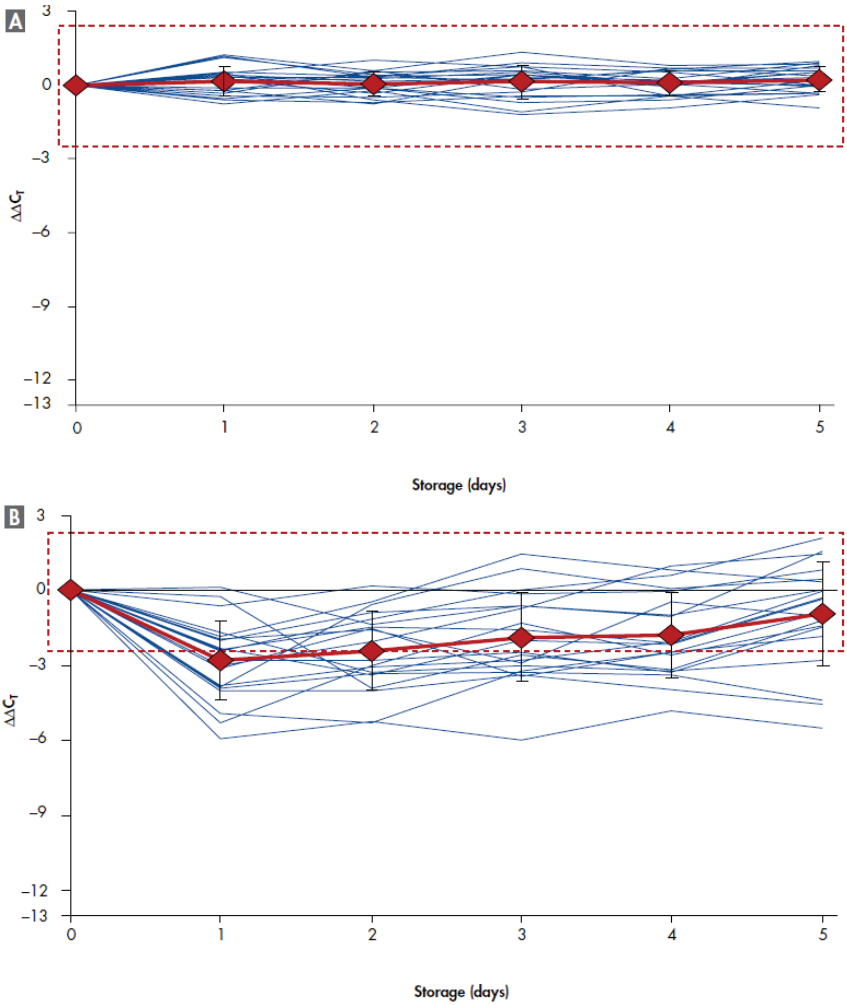


Figure 6: RNA stability in blood samples at 2–8°C: FOS. Blood was drawn from 10 donors, with duplicate samples and stored at 2–8°C for the indicated number of days, followed by total RNA isolation. [**A**] Blood was collected and stored in PAXgene Blood RNA Tubes (BRT), and total RNA was purified using the PAXgene Blood RNA Kit. [**B**] Blood was collected and stored in standard blood collection tubes with EDTA as an anticoagulant, and total RNA was purified using a standard organic isolation method with silica-membrane–based RNA cleanup. Relative transcript levels of FOS were determined by real-time, duplex RT-PCR, using 18S rRNA as an internal standard. The values for all samples are plotted, with means and standard deviations of all samples shown. The dashed lines indicate the ±3x total precision of the assay (2.34 C_T).

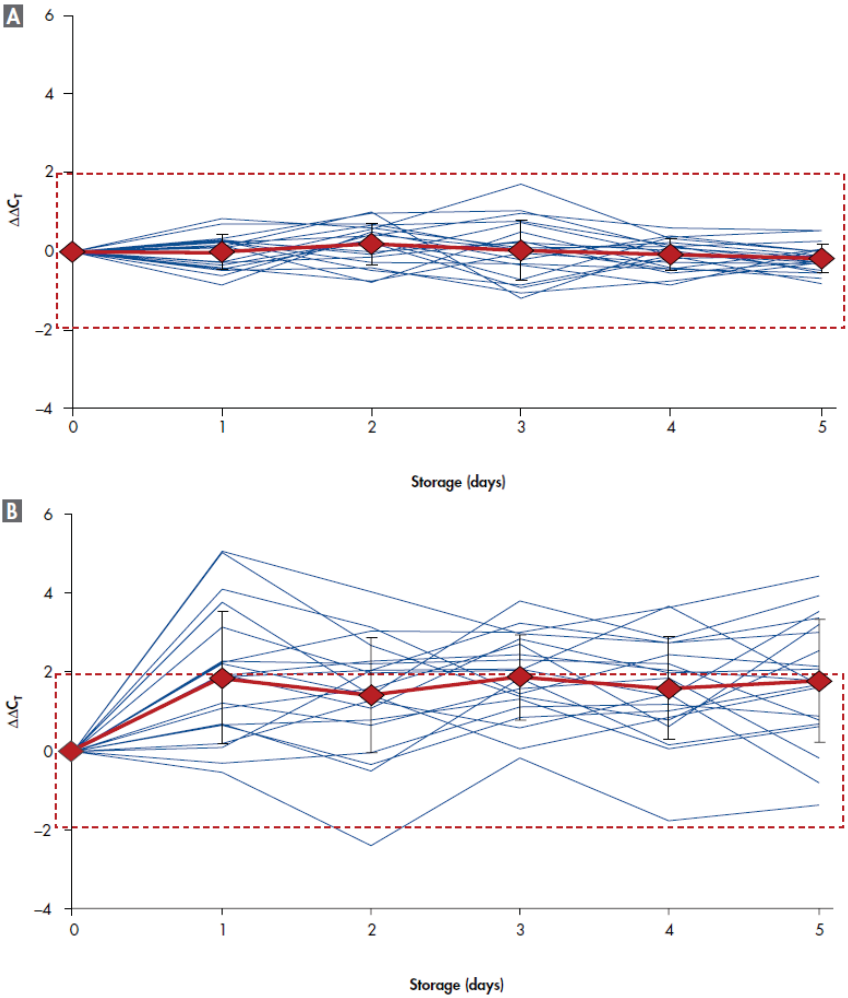


Figure 7: RNA stability in blood samples at 2–8°C: IL1B. Blood was drawn and total RNA purified, after storage at 2–8°C, as described in Figure 6. Relative transcript levels of IL1B were determined by real-time, duplex RT-PCR, using 18S rRNA as an internal standard. The values for all samples are plotted, with means and standard deviations of all samples shown. The dashed lines indicate the ±3x total precision of the assay (1.93 C_T).

Manual RNA isolation

Total RNA isolated using the PAXgene Blood RNA System is pure. Using the manual protocol, A_{260}/A_{280} values are between 1.8 and 2.2, and ≤1% (w/w) genomic DNA is present in ≥95% of all samples, as measured by quantitative, real-time PCR of a sequence of the beta-actin gene. At least 95% of samples show no inhibition in RT-PCR when the eluate accounts for up to 30% of the RT-PCR reaction volume.

Using the manual protocol, average sample preparation time (based on data from 12 sample preparation runs) is approximately 90 min*, with only 40 min of hands-on time. RNA yields from 2.5 ml healthy human whole blood are ≥3 µg for ≥95% of the samples processed. Since yields are highly donor-dependent, individual yields may vary. For individual donors, the PAXgene Blood RNA System provides highly reproducible and repeatable yields (Figure 8 and Figure 9, pages 44 and 45, respectively) and reproducible and repeatable RT-PCR (Figure 10 and Figure 11, pages 49 and 50, respectively), making it highly robust for clinical diagnostic tests.

Figure 8 (page 44) indicates the overall repeatability and reproducibility of the PAXgene Blood RNA System. Additional studies were conducted to show the influence of different PAXgene Blood RNA kit lots and different operators on the reproducibility of RNA yield and real time RT-PCR performance. As pooled blood samples instead of individual PAXgene Blood RNA Tubes (BRT) were used for these studies, the results do not reflect the system repeatability, including fluctuation between individual blood draws, but only the repeatability of the sample preparation (see Figure 9, page 45).

* Total protocol runtime, including upfront handling of PAXgene Blood RNA Tubes (centrifugations, pellet wash and pellet resuspension).

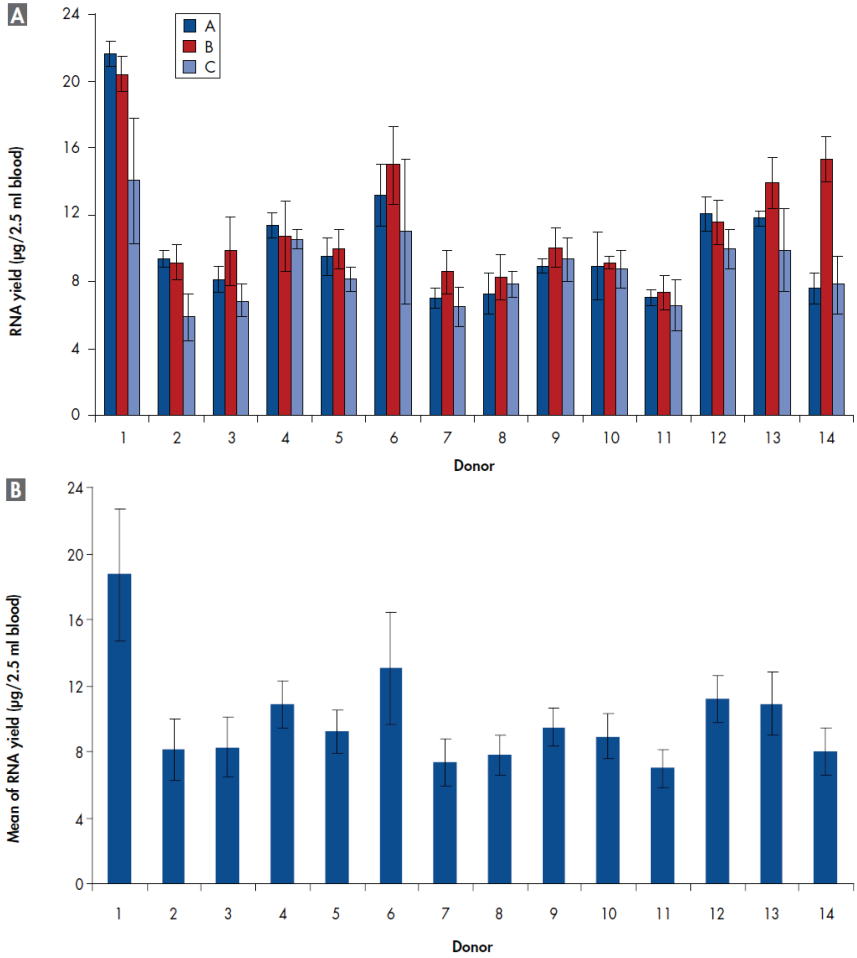


Figure 8: Reproducible and repeatable RNA isolation. Quadruplicate blood samples from 14 donors were manually processed by each of 3 technicians (A, B, C). Three sets of equipment were used, and all samples prepared by a single technician were processed using the same equipment. [**A**] Means and standard deviations of RNA yield per replicate samples from the same donors and different technicians are shown. [**B**] Twelve replicate blood samples from each of 14 donors were processed by the 3 different technicians. Means and standard deviations of RNA yield per samples from the same donors and all technicians are presented. For all RNA samples, A_{260}/A_{280} ratios ranged from 1.8 to 2.2.

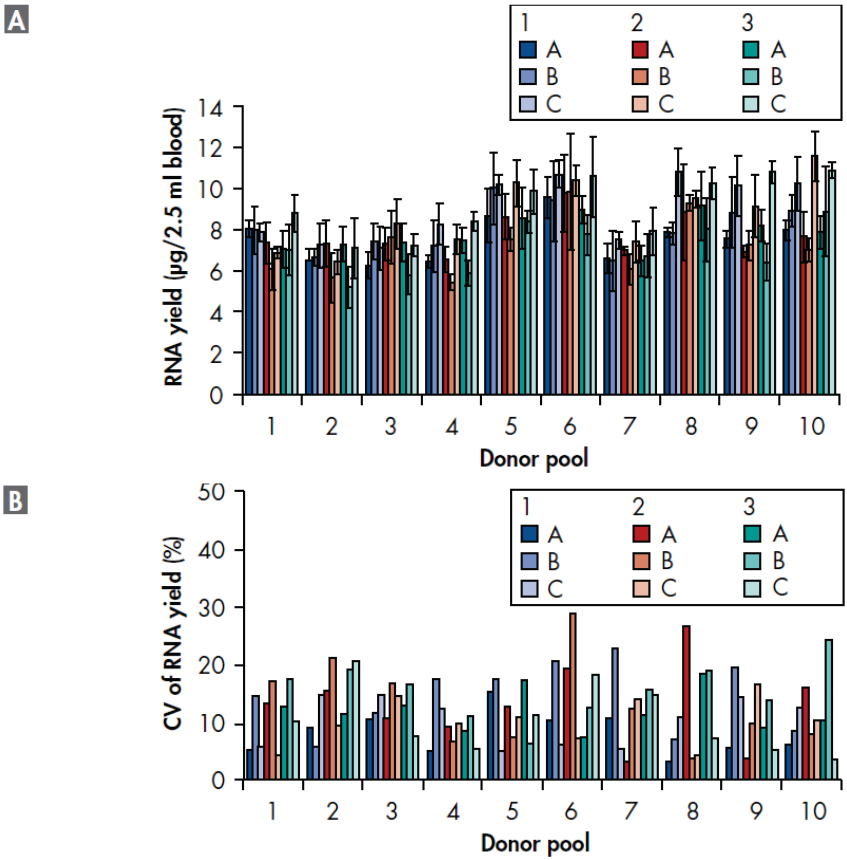


Figure 9: Repeatability and reproducibility of RNA yield for different operators and PAXgene Blood RNA Kit lots using pooled blood samples. Blood samples from 30 different donors were collected in PAXgene Blood RNA Tubes (BRT; 12 tubes per donor, 360 tubes in total). The contents of the tubes from 3 donors were pooled and subsequently realiquoted into 36 samples. These 36 samples per 3-donor-pool were manually processed by 3 different operators. Each operator used 3 different PAXgene Blood RNA Kit lots for the isolation of RNA and processed quadruplicate samples from each of the 10 donor pools. [**A**] RNA yield and standard deviation for every operator–lot combination. Quadruplicate blood samples from 10 donor pools were processed by 3 different operators (A, B, C) with each of 3 kit lots (1, 2, 3). The mean yields (columns) and standard deviations (error bars) per quadruplicate sample from the same donor pool for different operator and different kit lot are presented. [**B**] CV of RNA yield per donor pool for all operator–lot combinations (A, B, C; 1, 2, 3) as calculated from the mean yield and standard deviation of the yield shown in Figure 9A.

Table 1A: Reproducibility within each lot and within each user for selected donor pools (1, 6, 9, 10)

Combination of data	Donor pool 1 (5.1 x 10^6 cells/ml)			Donor pool 6 (6.5 x 10^6 cells/ml)		
	Mean yield (µg)	SD (µg)	CV (%)	Mean yield (µg)	SD (µg)	CV (%)
Lot 1, user A	8.03	0.42	5	9.55	0.99	10
Lot 1, user B	7.98	1.17	15	9.38	1.94	21
Lot 1, user C	7.87	0.45	6	10.71	0.65	6
Lot 2, user A	7.32	0.98	13	9.78	1.89	19
Lot 2, user B	6.09	1.04	17	9.82	2.83	29
Lot 2, user C	6.87	0.31	4	10.37	0.74	7
Lot 3, user A	7.04	0.90	13	8.96	0.68	8
Lot 3, user B	6.98	1.22	17	7.73	0.97	13
Lot 3, user C	8.78	0.89	10	10.59	1.94	18
	Donor pool 9 (8.4 x 10^6 cells/ml)			Donor pool 10 (10.2 x 10^6 cells/ml)		
	Mean yield (µg)	SD (µg)	CV (%)	Mean yield (µg)	SD (µg)	CV (%)
Lot 1, user A	7.52	0.41	6	7.96	0.49	6
Lot 1, user B	8.82	1.72	19	8.90	0.76	9
Lot 1, user C	10.14	1.46	14	10.22	1.29	13
Lot 2, user A	6.92	0.27	4	7.63	1.23	16
Lot 2, user B	7.20	0.71	10	7.00	0.56	8
Lot 2, user C	9.14	1.52	17	11.56	1.21	10
Lot 3, user A	8.18	0.76	9	7.85	0.82	10
Lot 3, user B	6.41	0.88	14	8.88	2.17	24
Lot 3, user C	10.78	0.56	5	10.88	0.37	3

Table 1B: Reproducibility within each user and between all lots for selected donor pools (1, 6, 9, 10)

Combination of data	Donor pool 1 (5.1 x 10^6 cells/ml)			Donor pool 6 (6.5 x 10^6 cells/ml)		
	Mean yield (µg)	SD (µg)	CV (%)	Mean yield (µg)	SD (µg)	CV (%)
User A, all lots	7.46	0.85	11	9.43	1.22	13
User B, all lots	7.02	1.31	19	8.98	2.09	23
User C, all lots	7.84	0.98	13	10.56	1.15	11
	Donor pool 9 (8.4 x 10^6 cells/ml)			Donor pool 10 (10.2 x 10^6 cells/ml)		
	Mean yield (µg)	SD (µg)	CV (%)	Mean yield (µg)	SD (µg)	CV (%)
User A, all lots	7.54	0.72	10	7.81	0.82	11
User B, all lots	7.48	1.50	20	8.26	1.54	19
User C, all lots	10.02	1.34	13	10.89	1.10	10

Table 1C: Reproducibility within each lot and between all users for selected donor pools (1, 6, 9, 10)

Combination of data	Donor pool 1 (5.1 x 10^6 cells/ml)			Donor pool 6 (6.5 x 10^6 cells/ml)		
	Mean yield (µg)	SD (µg)	CV (%)	Mean yield (µg)	SD (µg)	CV (%)
Lot 1, all users	7.96	0.69	9	9.88	1.34	14
Lot 2, all users	6.76	0.93	14	9.99	1.84	18
Lot 3, all users	7.60	1.27	17	9.09	1.71	19
	Donor pool 9 (8.4 x 10^6 cells/ml)			Donor pool 10 (10.2 x 10^6 cells/ml)		
	Mean yield (µg)	SD (µg)	CV (%)	Mean yield (µg)	SD (µg)	CV (%)
Lot 1, all users	8.83	1.63	19	9.02	1.27	14
Lot 2, all users	7.75	1.36	18	8.73	2.31	26
Lot 3, all users	8.46	1.99	24	9.20	1.80	20

Table 1D: Reproducibility between all lots and all users for selected donor pools (1, 6, 9, 10)

Combination of data	Donor pool 1 (5.1 x 10^6 cells/ml)			Donor pool 6 (6.5 x 10^6 cells/ml)		
	Mean yield (µg)	SD (µg)	CV (%)	Mean yield (µg)	SD (µg)	CV (%)
Lot 1, all users	7.44	1.09	15	9.66	1.65	17
	Donor pool 9 (8.4 x 10^6 cells/ml)			Donor pool 10 (10.2 x 10^6 cells/ml)		
	Mean yield (µg)	SD (µg)	CV (%)	Mean yield (µg)	SD (µg)	CV (%)
Lot 1, all users	8.35	1.70	20	8.99	1.80	20

Detailed analysis of 4 representative donor pools. The pools were selected according to the white blood cell count and reflect the upper, medium and the lower values of the normal range of white blood cell counts (4.8 x 10^6 – 1.1 x 10^7 leukocytes/ml). The white blood cell count represents the mean value of the 3 white blood cell counts from the 3 donors per donor pool.

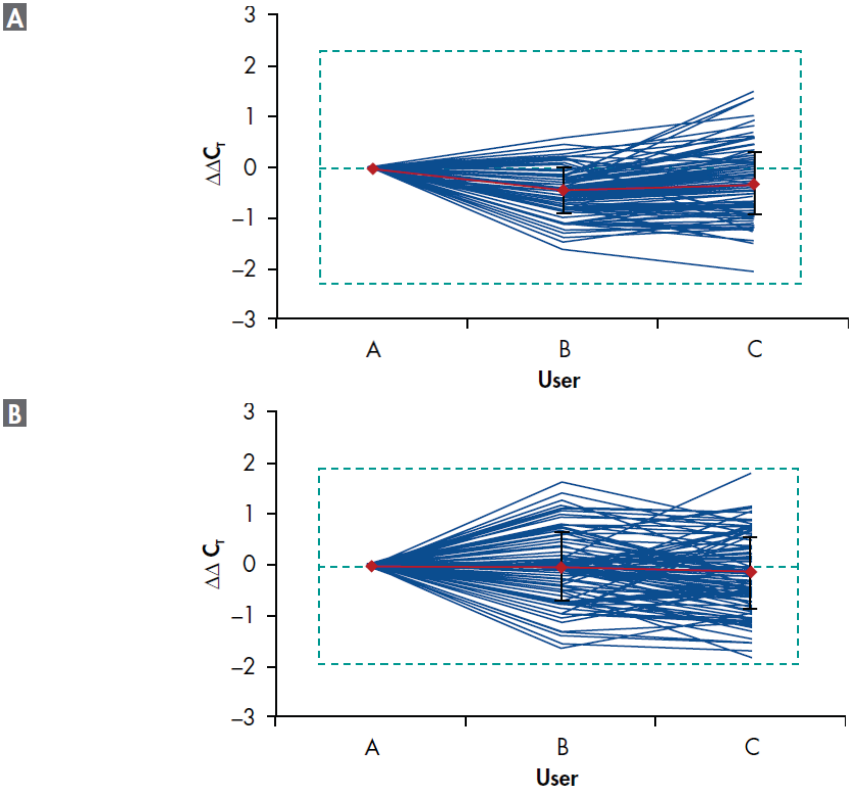


Figure 10: Reproducibility of RT-PCR — between users. RNA purified in the experiment described in Figure 9 was used for real-time RT-PCR. Relative transcript levels of [**A**] FOS and [**B**] IL1B were determined by real-time, duplex RT-PCR using 18S rRNA as an internal standard. The values for all samples are plotted, relative to the values for user A (10 donor pools x 3 kit lots x 4 replicates = 120 data sets for each gene), with means (red lines) and standard deviations (black bars) for all samples shown. The dashed lines indicate the ±3x total precision of the assays (FOS: 2.34 C_T; IL1B: 1.93 C_T).

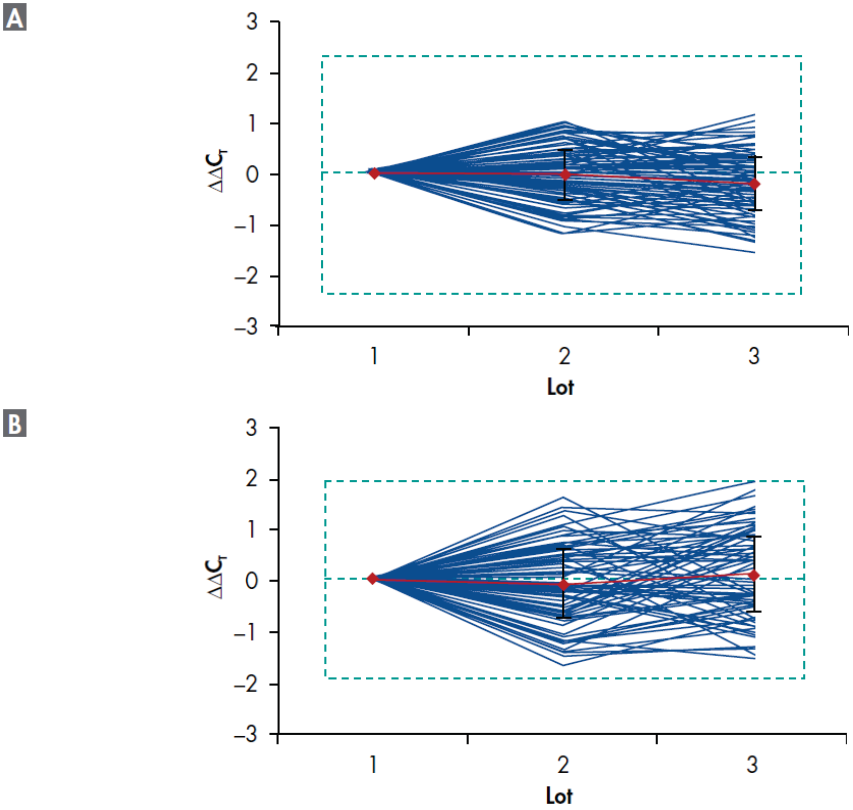


Figure 11: Reproducibility of RT-PCR — between kit lots. RNA purified in the experiment described in Figure 9 was used for real-time RT-PCR. Relative transcript levels of [**A**] FOS and [**B**] IL1B were determined by real-time, duplex RT-PCR using 18S rRNA as an internal standard. The values for all samples are plotted, relative to the values for kit lot 1 (10 donor pools x 3 users x 4 replicates = 120 data sets for each gene), with means (red lines) and standard deviations (black bars) for all samples shown. The dashed lines indicate the ±3x total precision of the assays (FOS: 2.34 C_T; IL1B: 1.93 C_T).

Table 2: Summary of RT-PCR data from Figure 10 and Figure 11

Test system	FOS/18S rRNA assay		IL1B/18S rRNA assay	
Comparison of data	**Mean ($\Delta\Delta C_T$)**	**± SD ($\Delta\Delta C_T$)**	**Mean ($\Delta\Delta C_T$)**	**± SD ($\Delta\Delta C_T$)**
Reproducibility within each user and between all lots				
All users, lot 1–lot 1	0.00	0.00	0.00	0.00
All users, lot 1–lot 2	–0.03	0.48	–0.07	0.66
All users, lot 1–lot 3	–0.21	0.52	0.11	0.71
Reproducibility within each user and between all lots				
All lots, user A–user A	0.00	0.00	0.00	0.00
All lots, user A–user B	–0.46	0.44	–0.06	0.69
All lots, user A–user C	–0.31	0.60	–0.15	0.71

User: Technician, performed the study.
Lot: Number of kit lot used in this study.
SD: Standard deviation.
Mean $\Delta\Delta C_T$ values (N = 120) and standard deviations are shown for the data presented in Figure 10 and Figure 11.

Automated RNA isolation

RNA yields from 2.5 ml healthy human whole blood are ≥3 µg for ≥95% of the samples processed. Figure 12 (page 52) indicates the RNA yields from a total of 216 samples prepared using the automated protocol with 3 kit lots by 3 operators. As pooled blood samples instead of individual PAXgene Blood RNA Tubes (BRT) were used for these studies, the results do not reflect the RNA yield expected from single samples of individual blood draws. Since yields are highly donor-dependent, individual yields may vary (Figure 12, page 52).

At least 95% of samples show no inhibition in RT-PCR when the eluate accounts for up to 30% of the RT-PCR reaction volume. Using the automated protocol, cross contamination between samples is undetectable, as measured by quantitative, real-time RT-PCR of sequences of the ABL1 and FOS transcripts in RNA-negative samples (water) paired with RNA-positive samples (human whole blood) in the same run.

RNA isolated with the PAXgene Blood RNA System and the automated protocol is pure, as shown by lack of RT-PCR inhibition and A_{260}/A_{280} values between 1.8 and 2.2. Genomic DNA is present at ≤1% (w/w) in ≥95% of all samples, as measured by quantitative, real-time PCR of a sequence of the beta-actin gene. Figure 13 and Figure **14** (page 53) show the A_{260}/A_{280} values and relative genomic DNA of a total of 216 samples prepared using the automated protocol with 3 kit lots by 3 operators.

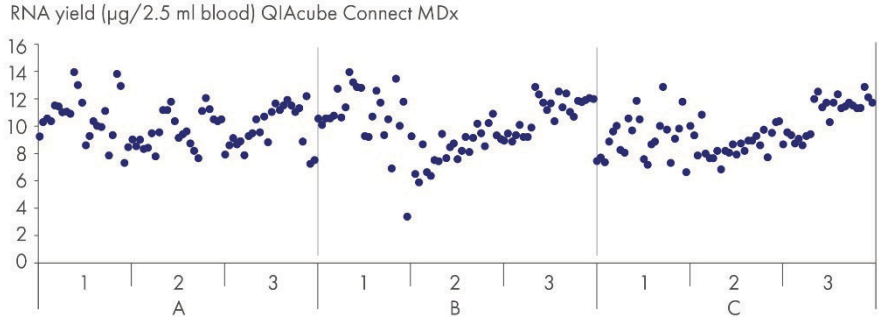


Figure 12: RNA yield — automated processing with QIAcube Connect MDx. Blood samples from individual donors were collected in PAXgene Blood RNA Tubes (BRT). The contents of the tubes were pooled into 6 donor pools and subsequently realiquoted. A total of 216 tubes (i.e., 36 per pool) were processed by 3 different operators (A, B, C). Each operator used 3 different lots (1, 2, 3) of the PAXgene Blood RNA Kit for automated isolation with QIAcube Connect MDx and processed quadruplicate samples from each of the 6 donor pools. RNA yields of all individual samples are shown for every operator–lot combination.

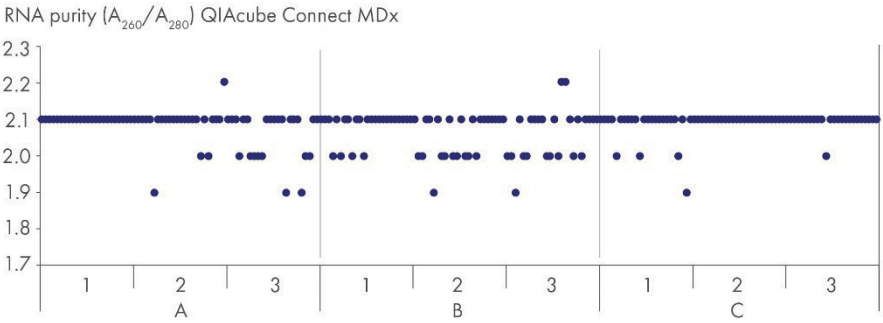


Figure 13: RNA purity (A_{260}/A_{280} values) — automated processing with QIAcube Connect MDx. RNA was purified by 3 different operators (A, B, C) using 3 different lots (1, 2, 3) of the PAXgene Blood RNA Kit with QIAcube Connect MDx in the experiment described in Figure 12. A_{260}/A_{280} values of all individual samples are shown for every operator–lot combination.

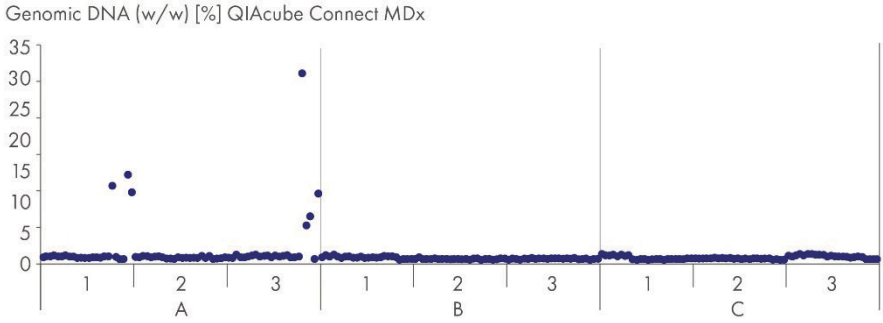


Figure 14: RNA purity (% genomic DNA contamination) — automated processing with QIAcube Connect MDx. RNA was purified by 3 different operators (A, B, C) using 3 different lots (1, 2, 3) of the PAXgene Blood RNA Kit with QIAcube Connect MDx in the experiment described in Figure 12. Genomic DNA amounts (w/w) in all individual samples are shown for every operator–lot combination.

The automated protocol of RNA isolation using the PAXgene Blood RNA System provides highly reproducible and repeatable RT-PCR results making it highly robust for clinical diagnostic tests.

Stability of isolated RNA

RNA samples isolated from blood filled PAXgene Blood RNA Tubes with the PAXgene Blood RNA Kit are stable for 5 years of storage at –20°C and 7 years of storage at –70°C (endpoint of studies).

Important Notes

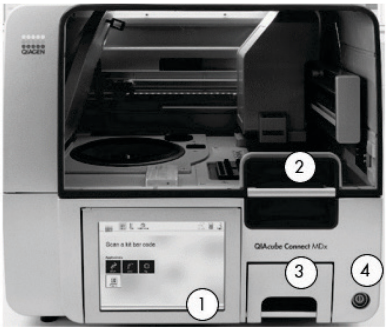
Using the QIAcube Connect MDx

Ensure that you are familiar with operating the QIAcube Connect MDx. Please read the instrument user manual and any additional information supplied with the instrument, paying careful attention to the safety information, before beginning the automated PAXgene Blood RNA protocol.

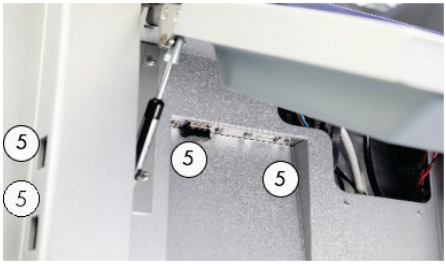
Starting the QIAcube Connect MDx

Close the QIAcube Connect MDx hood, and switch on the instrument with the power switch (see Figure 15, page 56).

A beeper sounds and the startup screen appears. The instrument automatically performs initialization tests.



Front view of the QIAcube Connect MDx



Pulled-out touchscreen

Rear view of QIAcube Connect MDx (left side)

Rear view of QIAcube Connect MDx (right side)

Figure 15: External features of the QIAcube Connect MDx.

1. Touchscreen
2. Hood
3. Waste drawer
4. Power switch
5. 2 USB ports on the left side of the touchscreen; 2 USB ports behind the touchscreen (Wi-Fi module plugged into 1 USB port)
6. RJ-45 Ethernet port
7. Power cord socket
8. Cooling air outlet

Touchscreen

The QIAcube Connect MDx is controlled using a touchscreen. The touchscreen allows the user to operate the instrument and guide the user through worktable setup. During sample processing, the touchscreen shows the protocol status and remaining time.

Figure 16: Pulled-out touchscreen of QIAcube Connect MDx.

Installing protocols on the QIAcube Connect MDx

An initial protocol installation may be required before the first RNA preparation run on the QIAcube Connect MDx can be performed. Install both "PAXgene Blood RNA Part A" and "PAXgene Blood RNA Part B" protocols.

Protocols for the QIAcube Connect MDx are provided at **www.qiagen.com** and need to be downloaded to the USB stick supplied with the instrument. These protocols will be transferred to the instrument via the USB port.

The USB port (located on the side of the touchscreen; see Figure 15, page 56) allows connection of the QIAcube Connect MDx to the USB stick supplied with the instrument. Data files, such as log files or report files, can also be transferred via the USB port from the instrument to the USB stick.

The USB port is only for use with the USB stick provided by QIAGEN. Do not connect other devices to this port.

Do not remove the USB stick while downloading protocols or transferring data files or during a protocol run.

For further details on the process of uploading protocols to the QIAcube Connect MDx, please see the user manual of the instrument.

Loading the QIAcube Connect MDx

To save time, loading can be performed during one or both of the 10 min centrifugation steps (steps 3 and 5) in "Protocol: Automated Isolation of Total RNA from Human Whole Blood Collected into PAXgene Blood RNA Tubes (BRT)", page 30.

Reagent bottles

Before every run on the QIAcube Connect MDx, carefully fill the 4 reagent bottles with the reagents listed in Table 3 (page 59) up to the maximum indicator level or, if that is not possible, to the level allowed by the buffer volumes supplied in the PAXgene Blood RNA Kit. Label the bottles and lids clearly with buffer names and place the filled reagent bottles into the appropriate positions on the reagent bottle rack. Load the rack onto the instrument worktable as shown (Figure 17 and Figure 18, pages 59 and 60, respectively).

The supplied volume of Buffer BR2 will not fill a reagent bottle to the indicator level. Buffers BR3 and BR4 may not fill the bottle to the indicator level after processing multiple samples in previous runs.

Be sure to remove lids from the bottles before placing onto the worktable.

Buffer volumes provided in the PAXgene Blood RNA Kit (50) are sufficient for a maximum of 7 RNA preparation runs on the QIAcube Connect MDx with 2 to 12 samples per run. In general, runs with a small number of samples per run should be avoided to process a total of 50 samples per kit. More than 7 RNA preparation runs can lead to insufficient buffer volumes for processing the last samples.

Table 3: Positions in the reagent bottle rack

Position	Reagent
1	Binding buffer (BR2)
2	Ethanol (96–100% v/v)
3	Wash buffer 1 (BR3)
4	Wash buffer 2 (BR4)*
5	– (leave empty)
6	– (leave empty)

* Wash buffer 2 (BR4) is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100% v/v, purity grade p.a.) as indicated on the bottle to obtain a working solution.

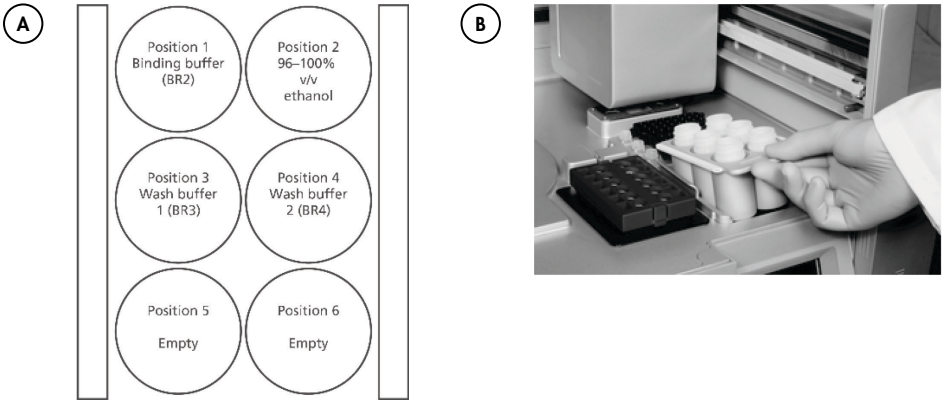


Figure 17: Loading the reagent bottle rack. [A] Schematic of positions and contents of bottles in the reagent bottle rack. **[B]** Loading the rack onto the QIAcube Connect MDx.

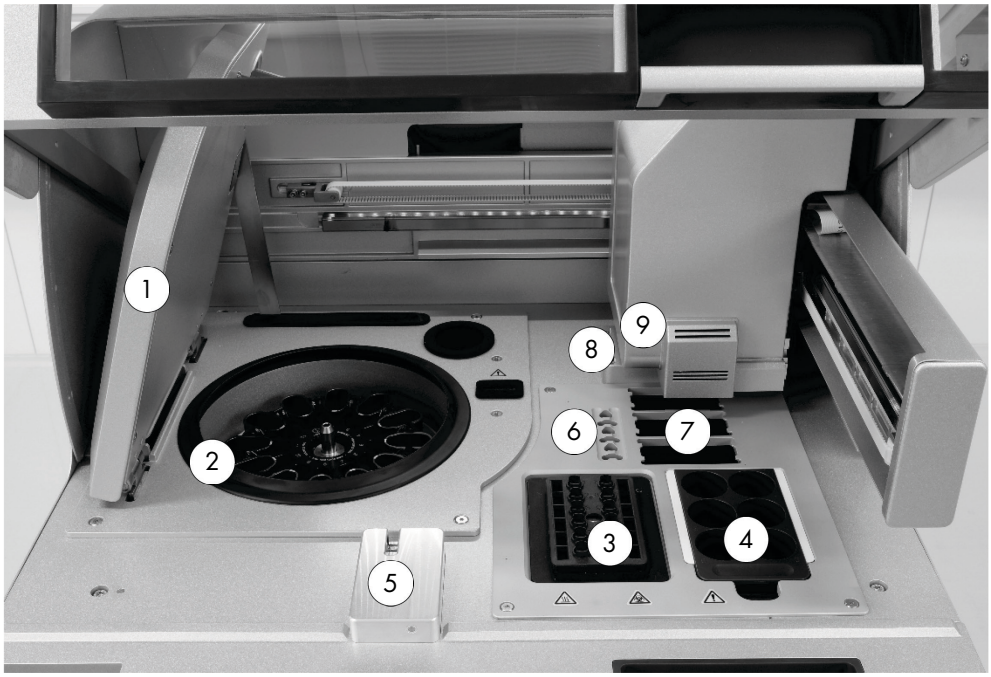


Figure 18: Internal view of the QIAcube Connect MDx.

1. Centrifuge lid
2. Centrifuge
3. Shaker
4. Reagent bottle rack
5. Tip sensor and hood lock
6. MCT slots
7. 3 slots for tip racks
8. Disposal slots for tips and columns
9. Robotic arm (includes 1 channel pipettor, gripper, ultrasonic and optical sensor and UV LED)

Spin columns (PSC, PRC), MCT and QIAcube Connect MDx plasticware

Place 2 tip racks filled with Filter-Tips 1000 µl onto the QIAcube Connect MDx (see Figure 18, page 60). Refill racks with tips when necessary.

Only use 1000 µl filter-tips designed for use with the QIAcube Connect MDx.

Label rotor adapters and MCT for each sample using a permanent pen. Open the PSC to be used, and cut the lid off completely using scissors (see Figure 19).

For proper operation of the QIAcube Connect MDx robotic gripper, completely remove (cut off) the lids and all plastic parts connecting the lid to the PSC (see Figure 19). Otherwise, the robotic gripper cannot grip the PSC properly.

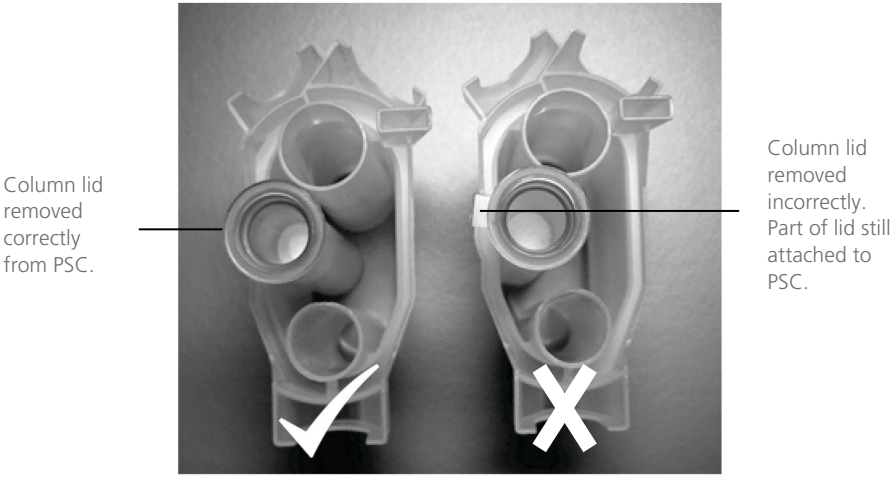


Figure 19: Loading the PSC. The PSC is loaded into the middle position of the rotor adapter. Cut off the lid of the PSC before loading the column.

Load the PSC (without lid, see Figure 19, page 61), PRC and labeled MCT into the appropriate positions in each labeled rotor adapter as shown in Table 4 and Figure 20.

Make sure that the spin column (PRC) and MCT lids are pushed all the way down to the bottom of the slots at the edge of the rotor adapter, otherwise the lids will break off during centrifugation.

Table 4: Plastic consumables in the rotor adapter

Position	Reagent	Lid position
1	PAXgene RNA spin column (red, PRC)	L1
2	PAXgene Shredder spin column (lilac, PSC) (cut off lid before placing in rotor adapter)	–
3	MCT*	L3

* Use the MCT (1.5 ml) included in the PAXgene Blood RNA Kit.

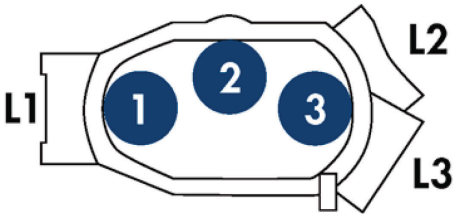


Figure 20: Positions in the rotor adapter. The rotor adapter has three tube positions (1–3) and three lid positions (L1–L3).

Loading the centrifuge

Load the assembled rotor adapters into the centrifuge buckets of the QIAcube Connect MDx as shown in Figure 21 below.

If processing fewer than 12 samples, make sure to load the centrifuge rotor balanced radially (see Figure 22, page 64). All centrifuge buckets must be mounted before starting a protocol run, even if fewer than 12 samples are to be processed. A single (one) sample or 11 samples cannot be processed.

Figure 21: Loading the centrifuge on the QIAcube Connect MDx. Load the assembled rotor adapters into the centrifuge buckets.

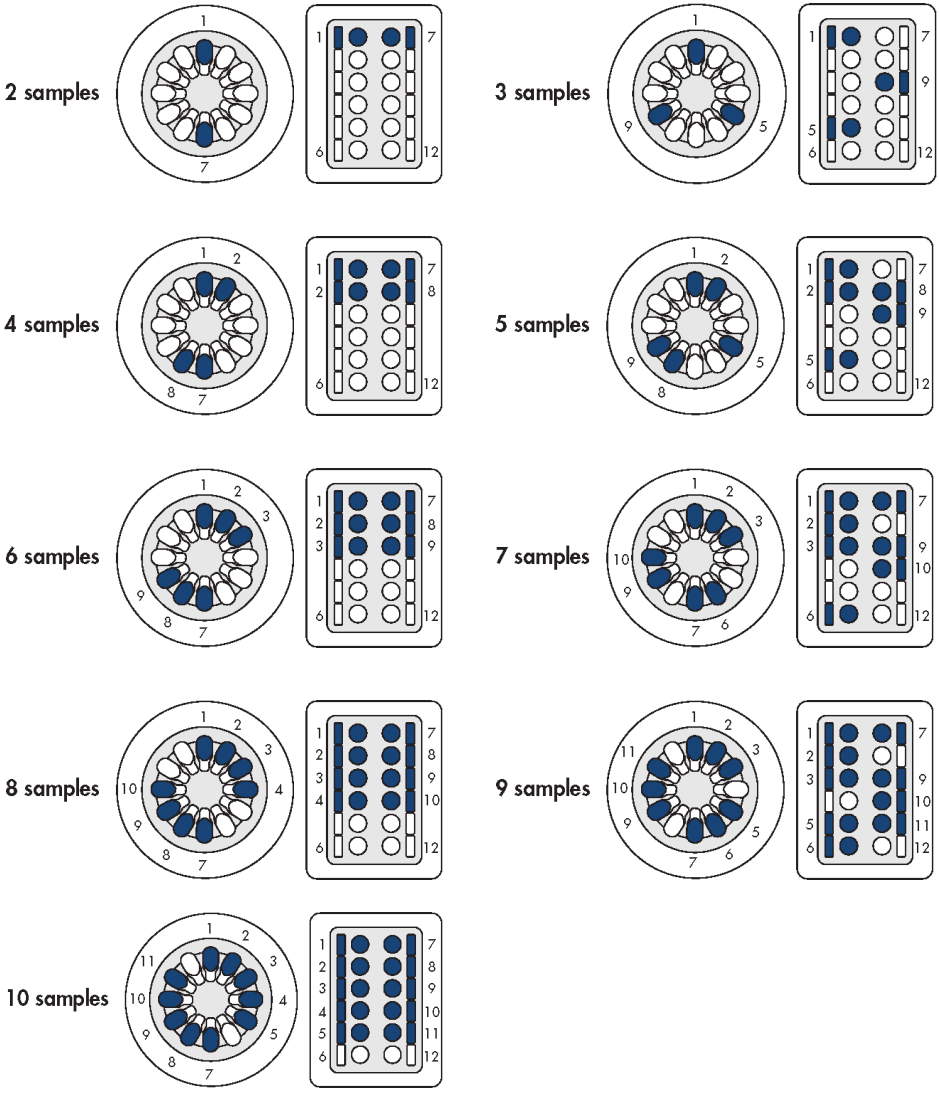


Figure 22: Loading the centrifuge and shaker. Centrifuge and shaker positions are shown for processing from two (2) to ten (10) samples. One (1) or 11 samples cannot be processed. For processing 12 samples, all centrifuge and shaker positions are loaded (image not shown).

Processing tubes

Remove any PTs left in the MCT slots from previous runs (see Figure 18, page 60). Fill 3 PTs with the amount of reagents given in Table 5 according to the number of samples in the run.

For DNase I incubation mix, pipet the indicated volume of DNA digestion buffer (RDD) into a PT and add the indicated volume of DNase I (RNFD) stock solution. Mix by gently pipetting the complete mixture up and down 3 times using a 1000 µl pipet tip.

Use the 2 ml PTs included in the PAXgene Blood RNA Kit. Label the tubes clearly with reagent names and place them into the appropriate position in the MCT slots, as indicated in Table 6 (page 66).

DNase I (RNFD) is especially sensitive to physical denaturation. Mix only by pipetting, using wide-bore pipet tips to reduce shearing. Do not vortex.

Be sure to only pipet the required volume as indicated in Table 5 below.

Table 5: Volume of reagents required in PTs for the MCT slots

Number of samples	Reagent volume for indicated number of samples (µl)		
	Proteinase K (PK)	DNase I incubation mix	Elution buffer (BR5)
2	126	187 (23 DNase I + 164 Buffer RDD)	313
3	170	261 (33 DNase I + 228 Buffer RDD)	399
4	213	334 (42 DNase I + 292 Buffer RDD)	486
5	256	407 (51 DNase I + 356 Buffer RDD)	572
6	299	481 (60 DNase I + 421 Buffer RDD)	658
7	342	554 (69 DNase I + 485 Buffer RDD)	745
8	386	627 (78 DNase I + 549 Buffer RDD)	831
9	429	701 (88 DNase I + 613 Buffer RDD)	918
10	472	775 (97 DNase I + 678 Buffer RDD)	1004
12	558	921 (115 DNase I + 806 Buffer RDD)	1177

Table 6: MCT slots

	Position		
	A	B	C
Content	Proteinase K	DNase I incubation mix	Elution buffer (BR5)
Vessel	Processing tube*	Processing tube*	Processing tube*

* Use the 2 ml PTs included in the PAXgene Blood RNA Kit.

Disposal

For safe disposal after specimen collection and manual RNA isolation, please refer to safety information and precautions on pages 17 and 18, respectively.

In addition, for automated RNA isolation using QIAcube Connect MDx, please refer to Figure 21 and Figure 22, pages 63 and 64, respectively, indicating dedicated slots of used tips and columns for disposal.

References

Rainen L., Oelmueller U, Jurgensen S, Wyrich R, Ballas C, Schram J, Herdman C, Bankaitis-Davis D, Nicholls N, Trollinger D, Tryon V (2002) Stabilization of mRNA expression in whole blood samples. Clin. Chem. 48, 1883-90.

Sambrook J and Russell D W (2001) Molecular Cloning: A Laboratory Manual, 3rd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

International Organization for Standardization (2019) Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for venous whole blood — Part 1: Isolated cellular RNA (ISO Standard No. 20186-1:2019).

Wilfinger W W, Mackey M, and Chomczynski P (1997) Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. BioTechniques 22, 474.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see 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
RNA degraded	
RNase contamination	Be careful not to introduce any RNases into the reagents during the procedure or later handling (see Appendix A, page 74).
Low RNA yield	
a) Less than 2.5 ml blood collected in PAXgene Blood RNA Tube (BRT)	Ensure that 2.5 ml blood is collected in the PAXgene Blood RNA Tube (BRT; see *PAXgene Blood RNA Tube Handbook*)
b) RNA concentration measured in water	RNA must be diluted in 10 mM Tris-HCl, pH 7.5* for accurate quantification (see Appendix B, page 75).
c) Cell debris transferred to PRC in steps 9 and 10 of the manual protocol	Avoid transferring large particles when pipetting the supernatant in step 7 of the manual protocol (transfer of small debris will not affect the procedure).
d) Supernatant not completely removed in step 3	Ensure the entire supernatant is removed. If the supernatant is decanted, remove drops from the rim of the PAXgene Blood RNA Tube (BRT) by dabbing onto a paper towel. Take appropriate precautions to prevent cross-contamination.
e) After collection into the PAXgene Blood RNA Tube (BRT), blood is incubated for less than 2 h	Incubate blood in the PAXgene Blood RNA Tube (BRT) for at least 2 h after collection.

* 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.

	Comments and suggestions
Low A_{260}/A_{280} value	
f) Water used to dilute RNA for A_{260}/A_{280} measurement	Use 10 mM Tris-HCl, pH 7.5 to dilute RNA before measuring purity* (see Appendix B, page 75).
g) Spectrophotometer not properly zeroed	Zero the spectrophotometer using a blank consisting of the same proportion elution buffer (BR5) and 10 mM Tris-HCl, pH 7.5, as in the samples to be measured. Elution buffer (BR5) has high absorbance at 220 nm, which can lead to high background absorbance levels if the spectrophotometer is not properly zeroed.
Instrument malfunction	
QIAcube Connect MDx did not operate properly	Read the *QIAcube Connect MDx User Manual*, paying careful attention to the Troubleshooting section. Make sure that the instrument is properly maintained, as described in the user manual.

* Wilfinger, W.W., Mackey, M., and Chomczynski, P. (1997) Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. BioTechniques 22, 474.

Symbols

The following symbols may appear in the instructions for use or on the packaging and labeling. Additional symbols are explained in Kit contents (page 5).

Symbol	Symbol definition
V<N1>	Version <N1> of the product
 <N2>	Contains reagents sufficient for <N2> tests
	Consult instructions for use
	Use by
IVD	In vitro diagnostic medical device
REF	Catalog number
LOT	Lot number
MAT	Material number
COMP	Components
NUM	Number
KU	Kunitz units
ADD	Adding
CONT	Contains
RCNS	Reconstituted

DNase	Deoxyribonuclease I
EtOH	Ethanol
GITC	Guanidine isothiocyanate
RNase-Free DNase Set	RNase-Free DNase Set
GTIN	Global Trade Item Number
	Temperature limitation
	Upper limit of temperature
	Manufacturer
EC REP	European authorized representative according to Regulation (EU) 2017/746
	Important note
	Addition of ethanol
CE	CE Mark. This product fulfills the requirements of the Regulation (EU) 2017/746 on in vitro diagnostic medical devices
UDI	Unique device identifier
	Caution
	WARNING: Hot surface

Contact Information

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of PreAnalytiX products. If you have any questions regarding the PAXgene Blood RNA Kit, please do not hesitate to contact us.

For technical assistance and more information, please see our Technical Support Center at **www.qiagen.com/Support**, call 00800-22-44-6000, or contact one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit **www.qiagen.com**).

Appendix A: General Remarks on Handling RNA

Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Because RNases are difficult to inactivate and even minute amounts are sufficient to degrade RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the isolation procedure. To create and maintain an RNase-free environment, precautions must be taken during pretreatment and use of disposable and non-disposable vessels and solutions while working with RNA.

General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep purified RNA on ice when aliquots are pipetted for downstream applications.

Protocols for removing RNase-contamination from glassware and solutions can be found in general molecular biology guides, such as Sambrook, J. and Russell, D. W. (2001) *Molecular Cloning: A Laboratory Manual*, 3rd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Appendix B: Quantification and Determination of Quality of Total RNA

Quantification of RNA

The concentration of RNA should be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer. To ensure significance, readings should be in the linear range of the spectrophotometer. An absorbance of 1 unit at 260 nm corresponds to 44 µg of RNA per ml (A_{260} = 1 ⇒ 44 µg/ml). This relation is valid only for measurements in 10 mM Tris-HCl, pH 7.5.* Therefore, if it is necessary to dilute the RNA sample and this should be done in 10 mM Tris-HCl. As discussed below (see "Purity of RNA", page 76), the ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity. When measuring RNA samples, be certain that cuvettes are RNase-free. Zero the spectrophotometer using a blank consisting of the same proportion elution buffer (BR5) and Tris-HCl buffer as in the samples to be measured. Elution buffer (BR5) has high absorbance at 220 nm, which can lead to high background absorbance levels if the spectrophotometer is not properly zeroed. An example of the calculation involved in RNA quantification is shown below.

Volume of RNA sample	=	80 µl
Dilution (1/15)	=	10 µl of RNA sample + 140 µl 10 mM Tris-HCl, pH 7.5
Measure absorbance of diluted sample in a cuvette (RNase-free).		
A_{260}	=	0.3
Concentration of sample	=	44 x A_{260} x dilution factor
	=	44 x 0.3 x 15
	=	198 µg/ml
Total yield	=	concentration x volume of sample in milliliters
	=	198 µg/ml x 0.08 ml
	=	15.8 µg RNA

* 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.

Purity of RNA

The ratio of the readings at 260 and 280 nm (A_{260}/A_{280}) provides an estimate of the purity of RNA with respect to contaminants that absorb in the UV, such as protein. However, the A_{260}/A_{280} ratio is influenced considerably by pH. Lower pH results in a lower A_{260}/A_{280} ratio and reduced sensitivity to protein contamination.* For accurate values, we recommend measuring absorbance in 10 mM Tris-HCl, pH 7.5. Pure RNA has an A_{260}/A_{280} ratio of 1.8–2.2 in 10 mM Tris-HCl, pH 7.5. Zero the spectrophotometer using a blank consisting of the same proportion elution buffer (BR5) and Tris-HCl buffer as in the samples to be measured. Elution buffer (BR5) has high absorbance at 220 nm, which can lead to high background absorbance levels if the spectrophotometer is not properly zeroed.

* Wilfinger, W.W., Mackey, M., and Chomczynski, P. (1997) Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. BioTechniques 22, 474.

Appendix C: Handling PAXgene Blood RNA Tubes (BRT)

The following recommendations from BD may be helpful when handling PAXgene Blood RNA Tubes (BRT). See the *PAXgene Blood RNA Tube Handbook* for more information about PAXgene Blood RNA Tubes (BRT).

Instructions for removal of BD Hemogard Closure

1. Grasp the PAXgene Blood RNA Tube (BRT) with one hand, placing the thumb under the BD Hemogard closure. (For added stability, place arm on solid surface.) With the other hand, twist the BD Hemogard closure while simultaneously pushing up with the thumb of the other hand **only until the tube stopper is loosened.**
2. Move thumb away before lifting closure. **Do not** use thumb to push closure off the PAXgene Blood RNA Tube (BRT). **Caution**: If the PAXgene Blood RNA Tube (BRT) contains blood, an exposure hazard exists. To help prevent injury during closure removal, it is important that the thumb used to push upward on the closure be removed from contact with the PAXgene Blood RNA Tube (BRT) as soon as the BD Hemogard closure is loosened.
3. Lift closure off the PAXgene Blood RNA Tube (BRT). In the unlikely event of the plastic shield separating from the rubber stopper, **do not reassemble closure.** Carefully remove rubber stopper from the PAXgene Blood RNA Tube (BRT).

Instructions for insertion of Secondary BD Hemogard Closure

1. Replace closure over the PAXgene Blood RNA Tube (BRT).
2. Twist and push down firmly until stopper is fully reseated. Complete reinsertion of the stopper is necessary for the closure to remain securely on the PAXgene Blood RNA Tube (BRT) during handling.

Ordering Information

Product	Contents	Cat. no.
PAXgene Blood RNA Kit (50)	50 PAXgene Spin Columns, 50 Shredder Spin Columns, Processing Tubes, RNase-Free DNase I, RNase-Free Reagents and Buffers. To be used in conjunction with the PAXgene Blood RNA Tubes	762174
PAXgene Blood RNA Tubes (100)	100 blood collection tubes	762165
Related Products that can be ordered from QIAGEN for RNA isolation automated on QIAcube		
Starter Pack, QIAcube	Pack includes: reagent bottle racks (3); rack labeling strips (8); 200 µl filter-tips (1024); 1000 µl filter-tips (1024); 1000 µl filter-tips, wide-bore (1024); 30 ml reagent bottles (18); rotor adapters (240); rotor adapter holder	990395
Filter-Tips, 1000 µl (1024)	Sterile, Disposable Filter-Tips, racked	990352
Reagent Bottles, 30 ml (6)	Reagent Bottles (30 ml) with lids; pack of 6; for use with the QIAcube reagent bottle rack	990393
Rotor Adapters (10 x 24)	For 240 preps: 240 Disposable Rotor Adapters; for use with QIAcube	990394
Reagent Bottle Rack	Rack for accommodating 6 x 30 ml reagent bottles on the QIAcube worktable	9026197
Rotor Adapter Holder	Holder for 12 disposable rotor adapters; for use with QIAcube	990392
Related products that can be ordered from BD for blood collection with PAXgene Blood RNA Tubes (BRT)*		
Blood Collection Set	BD Vacutainer® Safety-Lok™ 6 Blood Collection Set: 21G, 0.75 inch (0.8 × 19 mm) needle, 12 inch (305 mm) tubing with luer adapter; 50 per box, 200 per case	367286
BD Vacutainer One-Use Holder	Case only for 13 mm and 16 mm diameter; 1000/case	364815

Product	Contents	Cat. no.
BD Vacutainer Plus Serum Tubes	13 × 75 mm 4.0 ml draw with red BD Hemogard closure and paper label; 100/box, 1000/case	368975
BD Vacutainer EST Tube	13 × 75 mm 3.0 ml draw with clear BD Hemogard closure and see-through label; 100/box, 1000/case	362725
BD Vacutainer No Additive (Z) Tube	13 × 75 mm 3.0 ml draw with clear BD Hemogard closure and paper label; 100/box, 1000/case	366703

* These blood collection accessories represent typical products that can be used with PAXgene Blood RNA Tubes (BRT). To find out more about these accessories, including how to order, visit **www.preanalytix.com**.

Document Revision History

Date	Changes
04/2022	Initial IVDR release

Notes

Notes

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

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