February 2026

PAXgene® Blood miRNA Kit Handbook

For manual or automated purification of miRNA from whole blood

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

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

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Kit Contents

PAXgene Blood miRNA Kit Catalog no. Number of preps	(50) 763134 50
PAXgene RNA Spin Columns (red)	50
PAXgene Shredder Spin Columns (lilac)	50
Microcentrifuge Tubes (1.5 mL)	3 × 50 1 × 10
Processing Tubes (2 mL)	6 x 50
Buffer BM1 (resuspension buffer)	20 mL
Buffer BM2* (binding buffer)	18 mL
Buffer BM3*† (wash buffer concentrate)	15 mL
Buffer BM4† (wash buffer concentrate)	11 mL
Buffer BR5 (elution buffer)	6 mL
RNase-free water	2 × 125 mL
Proteinase K	2 × 1.4 mL
RNase-Free DNase Set: • RNase-Free DNase I (lyophilized) • Buffer RDD (DNA digestion buffer) • RNase-Free Water	 1500 units‡ 2 × 2 mL 2.0 mL
Secondary Hemogard® Closures	50

* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 6 for Safety Information.

† Before using for the first time, add the required volume of ethanol (96–100%) as indicated on the bottle to obtain a working solution.

‡ 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).

Shipping and Storage

The PAXgene Blood miRNA Kit is shipped at ambient temperature. The RNase-Free DNase Set box (containing RNase-free DNase, Buffer RDD and RNase-free water) should be stored immediately upon receipt at 2–8°C. The remaining components of the kits should be stored dry at room temperature (15–25°C). When stored properly, the kit is stable until the expiration date on the kit box.

Product Use Limitations

For Research Use Only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention or treatment of a disease. The performance characteristics of this product have not been fully established.

QIAcube® Connect is designed to perform fully automated purification of nucleic acids and proteins in molecular biology applications. The system is intended for use by professional users trained in molecular biological techniques and the operation of QIAcube Connect.

All due care and attention should be exercised in the handling of the products. We recommend all users of PreAnalytiX products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments or to other applicable guidelines like ISO 20186-1:2019.

The PAXgene Blood miRNA Kit is designed for use with human whole blood containing 4.8×10^6–1.1×10^7 leukocytes/mL.

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 QIAGEN or PreAnalytiX kit and kit component.



CAUTION: Do not add bleach or acidic solutions directly to the sample-preparation waste.

Buffer BM2 and Buffer BM3 contain guanidine thiocyanate. Guanidine salts can form highly reactive compounds when combined with bleach. If liquid containing these buffers 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.

If spilled, the RNA stabilizing solution and blood mixture in a PAXgene Blood RNA Tube can be disinfected with 1 volume commercial bleach solution (5% sodium hypochlorite) per 9 volumes RNA stabilizing solution and blood mixture.

Waste from sample preparation, such as supernatants from centrifugation steps of the purification procedure, is potentially infectious. Waste must be autoclaved or incinerated to destroy infectious material and then disposed according to official regulations.

Quality Control

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

Introduction

The PAXgene Blood miRNA System provides a complete solution for stabilization and purification of high-quality total RNA >18 nucleotides (including miRNA) from human whole blood. Blood is collected and stabilized in PAXgene Blood RNA Tubes. Total RNA >18 nucleotides (including miRNA) is then purified using the PAXgene Blood miRNA Kit.

The purified total RNA is ready to use and is ideally suited for downstream applications, including:

- RT-PCR and real-time RT-PCR
- cDNA synthesis
- Next-generation sequencing applications

The PAXgene Blood miRNA Kit allows the parallel processing of multiple samples either manually or on the QIAcube Connect (also see page 12). The PAXgene Blood miRNA procedure replaces traditional methods involving the use of toxic substances such as phenol and/or chloroform or time-consuming and tedious methods such as alcohol precipitation.

Important: The PAXgene Blood miRNA Kit can only be used in conjunction with PAXgene Blood RNA Tubes. The tubes are not included in the kits and are available from BD and BD-authorized distributors (cat. no. 762165, see page 52 for Ordering Information).

Principle and procedure

Blood samples are collected in PAXgene Blood RNA Tubes that contain a proprietary reagent. This reagent lyses blood cells and immediately stabilizes intracellular RNA

to preserve the gene expression profile. RNA stabilization is critical for reliable downstream gene expression analysis. Without stabilization, degradation of RNA and upregulation or downregulation of transcripts can occur immediately after blood is drawn.

Total RNA >18 nucleotides (including miRNA) is purified from the stabilized blood samples using well-established PAXgene silica-membrane technology. The PAXgene Blood RNA Tubes are first centrifuged to pellet the samples, which are then washed with water and resuspended in Buffer BM1. After digestion in Buffer BM2 with proteinase K, the samples are homogenized by centrifugation through PAXgene Shredder spin columns. Isopropanol is added to the samples to optimize binding conditions, and the samples are then centrifuged through PAXgene RNA spin columns, where total RNA >18 nucleotides (including miRNA) binds to the PAXgene silica membrane. The bound RNA is subjected to DNase digestion to remove genomic DNA contamination and washed with Buffer BM3 followed by Buffer BM4. Pure RNA is then eluted in Buffer BR5.

The procedure is simple and can be automated or performed manually (see Figure 1 and Figure 2).

With the PAXgene Blood miRNA Kit, all RNA molecules longer than 18 nucleotides are purified. The purified RNA includes both mRNA and small RNAs such as miRNA. Using the manual or automated protocol, A_{260}/A_{280} values lie between 1.8 and 2.2, and based on quantitative real-time PCR on a sequence of the beta-actin gene, ≤1% (w/w) genomic DNA is present in ≥95% of all samples. At least 95% of samples show no inhibition of RT-PCR when using up to 30% of the eluate volume. RNA yields from 2.5 mL healthy human whole blood are ≥3 µg for ≥95% of samples processed. Yields are strongly sample dependent and may vary. For individual samples, the PAXgene Blood RNA system provides highly reproducible and repeatable yields. Typical yields per tube are in the range of 6–8 µg.*

* See Technical Note "Typical total RNA yields from PAXgene Blood RNA Tubes processed with the PAXgene Blood miRNA Kit" at **www.preanalytix.com**.

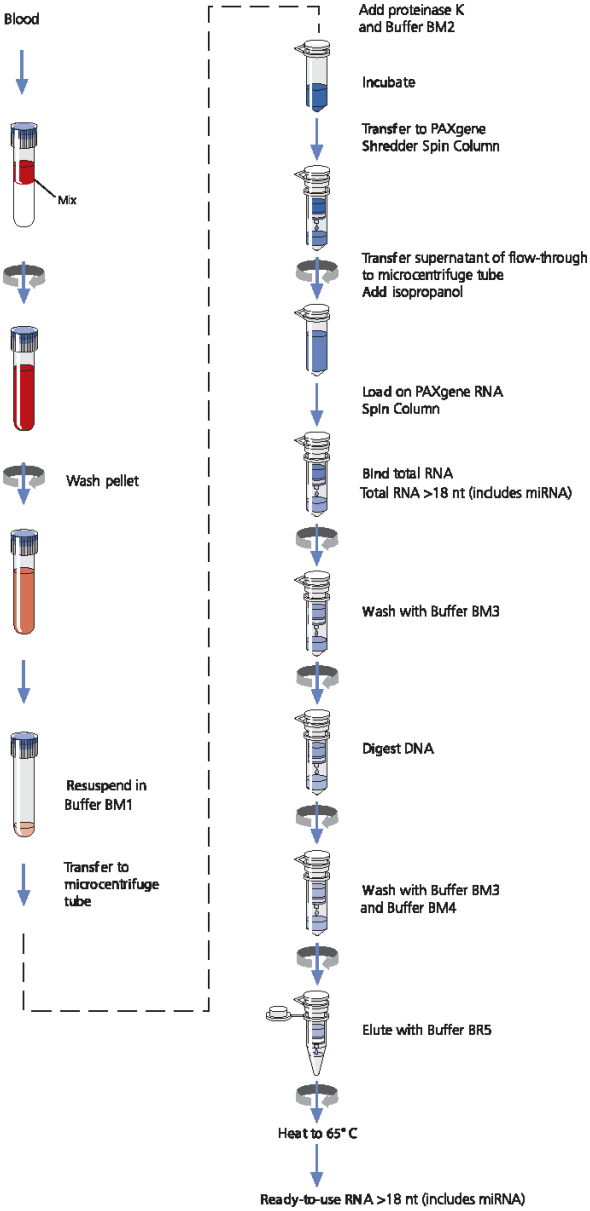


Figure 1. The manual PAXgene Blood miRNA procedure

Automated purification of total RNA, including miRNA, on QIAcube instruments

Purification of total RNA, including miRNA, can be fully automated on the QIAGEN QIAcube Connect, the QIAGEN QIAcube Connect MDx (Life Science Mode), or the classic QIAGEN QIAcube (hereafter called QIAcube). The innovative QIAcube instruments use advanced technology to process QIAGEN spin columns, enabling seamless integration of automated sample prep into your laboratory workflow. Sample preparation using QIAcube instruments follows the same steps as the manual procedure (i.e., lyse, bind, wash, and elute), enabling you to continue using the PAXgene Blood miRNA Kit for purification of high-quality total RNA, including miRNA.

QIAcube instruments are preinstalled with protocols for purification of plasmid DNA, genomic DNA, RNA, viral nucleic acids, and proteins, plus DNA and RNA cleanup. The range of protocols available is continually expanding, and additional QIAGEN protocols can be downloaded free of charge at **www.qiagen.com**.



QIAcube Connect

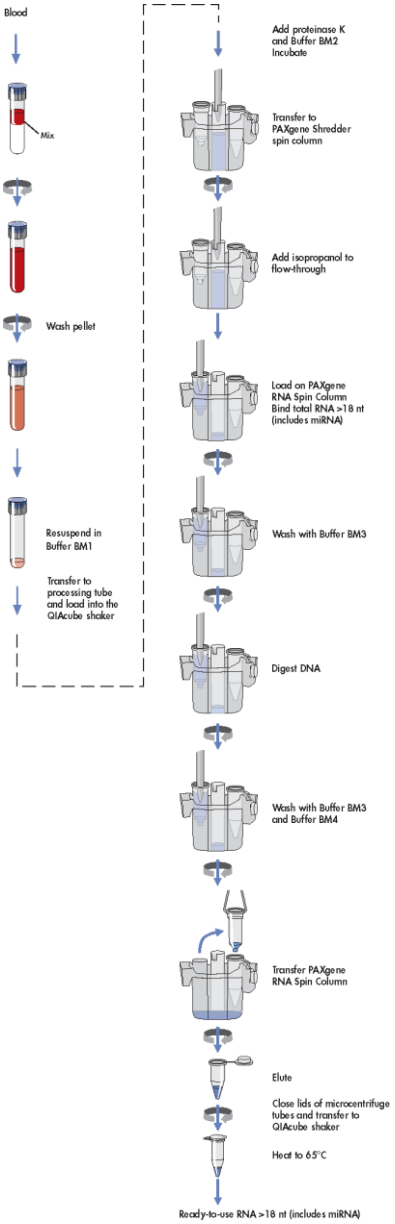


Figure 2. The automated PAXgene Blood miRNA procedure

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.

For all protocols

- PAXgene Blood RNA Tubes (BD and BD-authorized distributors, cat. no. 762165)
- Ethanol (96–100%, purity grade p.a.). Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.
- Isopropanol (100%, purity grade p.a.)
- Pipets (10 µL – 4 mL)*
- Sterile, aerosol-barrier, RNase-free pipet tips
- Graduated cylinder
- Centrifuge* capable of attaining 1000–8000 x *g* and equipped with a swing-out rotor and buckets to hold PAXgene Blood RNA Tubes
- Vortex mixer*
- Crushed ice
- Permanent pen for labeling

For the manual protocol

- Variable speed microcentrifuge† capable of attaining 8000 -20,000 x *g* and equipped with a rotor for 2 mL microcentrifuge tubes

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

† This is not a complete list of suppliers and does not include many important vendors of biological supplies.

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

For the automated protocol using QIAcube, QIAcube Connect, or QIAcube Connect MDx

- Scissors
- QIAcube instrument 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 instrument accessories
  - Rotor Adapter Holder (QIAGEN, cat. no. 990392)†

For the automated protocol using QIAcube Connect

- QIAcube Connect* (QIAGEN, cat.no. 9002864)
- QIAcube Connect service bundles:
  - QIAcube Connect System FUL-3 (QIAGEN, cat.no. 9002870)
  - QIAcube Connect System FUL-2 (QIAGEN, cat.no. 9002873)
  - QIAcube Connect System PRV-1 (QIAGEN, cat.no. 9002871)
  - QIAcube Connect System PRM-1 (QIAGEN, cat. no. 9245209)
  - QIAcube Connect, Core Agreement (QIAGEN, cat.no. 9245260)

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

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

For the automated protocol using QIAcube Connect MDx

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

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

Protocol: Manual Purification of Total RNA, Including miRNA, 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 damaged kit.
- When using a pipette, 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 processing tubes, microcentrifuge tubes, and rotor adapters are properly labeled using a permanent pen. Label the lid and the body of each microcentrifuge tube, the body of each processing tube, 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 pipette the sample into the bottom of the tube without moistening the rim of the tube.
- Always change pipette tips between liquid transfers. Use aerosol-barrier pipette tips.
- Avoid touching the spin column membrane with the pipette tip.

- After vortexing or heating a microcentrifuge tube, 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 according to the instructions in the *PAXgene Blood RNA Tube Handbook*.
- Ensure that the PAXgene Blood RNA Tubes are incubated for at least 2 h at room temperature after blood collection to ensure complete lysis of blood cells. Incubation of the PAXgene Blood RNA Tube 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 to room temperature and then incubate it at this temperature for 2 h before starting the procedure.
- Read the Safety Information on page 6.
- Read the guidelines on handling RNA (Appendix A, page 34).
- Ensure that instruments such as pipettes have been checked and calibrated regularly according to the manufacturer's recommendations.
- Buffers BM2 and BM3 may form a precipitate upon storage. If necessary, warm to 37°C to dissolve.
- Buffers BM3 and BM4 are supplied as a concentrate. Before using for the first time, add the appropriate volume of ethanol (96–100%, 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 (1500 Kunitz units)* in 550 µL of RNase-free

* 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).

water provided with the set. Take care that no DNase I is lost when opening the vial. Do not vortex the reconstituted DNase I. DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the vial.

- Current data show that reconstituted DNase I can be stored at 2–8°C for up to 6 weeks. For long-term storage of DNase I, remove the stock solution from the glass vial, divide it into single-use aliquots (use the 1.5 mL microcentrifuge tubes supplied with the kit; there are enough tubes for 5 aliquots), and store at –20°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.
- When reconstituting and aliquoting DNase I, ensure that you follow the guidelines for handling RNA (Appendix A, page 34).

Procedure

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

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

   Note: 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 to the pellet, and close the tube using a fresh secondary Hemogard closure (provided).

   If decanting the supernatant, 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 × *g* using a swing-out rotor. Remove the entire supernatant by decanting or pipetting, and discard.

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

Note: 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 Buffer BM1, and vortex until the pellet is visibly dissolved.
5. Pipet the sample into a 1.5 mL microcentrifuge tube. Add 300 µL Buffer BM2 and 40 µL proteinase K. Mix by vortexing for 5 s and incubate for 10 min at 55°C in a shaker–incubator at 400–1400 rpm. After incubation, set the temperature of the shaker–incubator to 65°C for use in step 20.

   Note: Do not mix Buffer BM2 and proteinase K together before adding them to the sample.
6. Pipet the sample into a PAXgene Shredder spin column (lilac) placed in a 2 mL processing tube, and centrifuge for 3 min at full speed (do not exceed 20,000 × *g*).
7. Carefully transfer the entire supernatant of the flow-through from the PAXgene Shredder spin column to a new 1.5 mL microcentrifuge tube without disturbing the pellet in the processing tube.
8. Add 700 µL of isopropanol (100%, purity grade p.a.), and mix by vortexing.
9. Pipet 700 µL sample into the PAXgene RNA spin column (red) placed in a 2 mL processing tube. Close the lid gently, and centrifuge for 1 min at 8000–20,000 × *g*.

   Place the spin column in a new 2 mL processing tube and discard the old processing tube containing the flow-through.*
10. Pipette the remaining sample into the PAXgene RNA spin column (red). Close the lid gently, and centrifuge for 1 min at 8000–20,000 × *g*. Place the spin column in a

* The flow-through contains Buffer BM2 or Buffer BM3 and is therefore not compatible with bleach. See page 7 for Safety Information.

new 2 mL processing tube, and discard the old processing tube containing the flow-through.*

11. Add 350 µL Buffer BM3 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 15 s at 8000–20,000 × *g*. Place the spin column in a new 2 mL processing tube, and discard the old processing tube containing the flow-through.*

    Note: Buffer BM3 is supplied as a concentrate. Ensure that ethanol is added to Buffer BM3 before use (see "Things to do before starting").

12. Add 10 µL DNase I stock solution to 70 µL Buffer RDD in a 1.5 mL microcentrifuge tube. Mix by gently flicking the tube, and centrifuge briefly to collect residual liquid from the sides of the tube.

    Note: 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 incubation mix (80 µL) directly onto the PAXgene RNA spin column membrane, and incubate on the benchtop (20–30°C) for 15 min.

    Note: Ensure that DNase I 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 O-ring of the spin column.

14. Add 350 µL Buffer BM3 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 15 s at 8000–20,000 × *g*. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing the flow-through.*

15. Add 500 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 15 s at 8000–20,000 × *g*. Discard the flow-through. Place the spin column in a new 2 mL processing tube and discard the old processing tube containing the flow-through.

* The flow-through contains Buffer BM2 or Buffer BM3 and is therefore not compatible with bleach. See page 7 for Safety Information.

Note: Buffer BM4 is supplied as a concentrate. Ensure that ethanol is added to Buffer BM4 before use (see "Things to do before starting").

16. Add another 500 µL Buffer BM4 to the PAXgene RNA spin column. Close the lid gently, and centrifuge for 2 min at 8000–20,000 × *g*.

    Note: After centrifugation, carefully remove the PAXgene RNA spin column from the processing tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.

17. Discard the processing tube containing the flow-through and place the PAXgene RNA spin column in a new 2 mL processing tube (supplied). Centrifuge at 8000–20,000 × *g* for 1 min.

    It is important to dry the spin column membrane since residual ethanol may interfere with downstream reactions.

18. Discard the processing tube containing the flow-through. Place the PAXgene RNA spin column in a new 1.5 mL microcentrifuge tube, and pipet 40 µL Buffer BR5 directly onto the spin column membrane. Close the lid gently and centrifuge for 1 min at 8000–20,000 × *g* to elute the RNA.

    Be sure to add Buffer BR5 directly to the spin column membrane. This wets the entire membrane, ensuring maximum elution efficiency.

19. Repeat the elution step (step 18) as described, using 40 µL Buffer BR5 and the same microcentrifuge tube.

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

    This incubation at 65°C denatures the RNA for downstream applications. Do not exceed the incubation time or temperature.

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

    Since the RNA remains denatured after freezing and thawing, it is not necessary to repeat the incubation at 65°C.

See Appendix B, page 36, for information about quantifying RNA. QIAGEN offers a range of optimized, ready-to-use kits for RT-PCR and real-time PCR that can be used for specific quantification of purified RNA. For details, visit **www.qiagen.com.**

For accurate quantification of RNA by absorbance at 260 nm, we recommend diluting the sample 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 Buffer BR5 and Tris-HCl buffer as in the samples to be measured. Buffer BR5 has high absorbance at 220 nm, which can lead to high background absorbance levels if the spectrophotometer is not properly zeroed.

Note: For quantification in Tris-HCl buffer, use the following relationship:

$A_{260} = 1 \Rightarrow 44$ µg/mL. See Appendix B, page 35.

* 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 Purification of Total RNA, Including miRNA, 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 damaged kit.
- 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 processing tubes, microcentrifuge tubes, and rotor adapters are properly labeled using a permanent pen. Label the lid and the body of each microcentrifuge tube, the body of each processing tube, 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 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 membrane with the pipette tip.

- After vortexing or heating a microcentrifuge tube, 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 according to the instructions in the *PAXgene Blood RNA Tube Instructions for Use (Handbook)*.
- Ensure that the PAXgene Blood RNA Tubes are incubated for at least 2 h at room temperature after blood collection to ensure complete lysis of blood cells. Incubation of the PAXgene Blood RNA Tube overnight may increase yields. If the PAXgene Blood RNA Tube 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 6.
- Read the guidelines on handling RNA (Appendix A, page 34).
- Read the *QIAcube Instrument User Manual* and any additional information supplied with the QIAcube instrument, paying careful attention to the Safety Information (also see page 6).
- Ensure that instruments, such as pipets and the QIAcube instrument, have been checked and calibrated regularly according to the manufacturer's recommendations.
- Buffers BM2 and BM3 may form a precipitate upon storage. If necessary, warm to 37°C to dissolve.
- Buffers BM3 and BM4 are supplied as a concentrate. Before using for the first time, add the appropriate volume of ethanol (96–100%, 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 (1500 Kunitz units)* in 550 µL of RNase-free water provided with the set. Take care that no DNase I is lost when opening the vial. Do not vortex the reconstituted DNase I. DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the vial.
- Current data show that reconstituted DNase I can be stored at 2–8°C for up to 6 weeks. For long-term storage of DNase I, remove the stock solution from the glass vial, divide it into single-use aliquots (use the 1.5 mL microcentrifuge tubes supplied with the kit; there are enough for 5 aliquots), and store at –20°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.
- When reconstituting and aliquoting DNase I, ensure that you follow the guidelines for handling RNA (Appendix A, page 34).
- Install the correct shaker adapter (included with the QIAcube instrument; 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 the protocols if not already done for previous runs. Install both "PAXgene Blood miRNA Part A" and "PAXgene Blood miRNA Part B" protocols. See "Installing protocols on the QIAcube instrument", page 42.

* Kunitz units are the commonly used units for measuring DNase I, defined as the amount of DNase I that causes an increase in A260 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).

Procedure

1. Close the QIAcube hood, and switch on the QIAcube with the power switch (see Figure 3, page 41).
2. A beeper sounds and the startup screen appears. The instrument automatically performs initialization tests.
3. Open the QIAcube hood and load the necessary reagents and plasticware into the QIAcube. See "Loading the QIAcube ", page 42.

   To save time, loading can be performed during one or both of the following 10 min centrifugation steps (steps 4 and 6).
4. Centrifuge the PAXgene Blood RNA Tube for 10 min at 3000–5000 × *g* using a swingout rotor.

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

   Note: The rotor must contain tube adapters for round-bottom tubes. If other types of tube adapter are used, the tubes may beak during centrifugation.
5. Remove the supernatant by decanting or pipetting. Add 4 mL RNase-free water 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.
6. Vortex until the pellet is visibly dissolved, and centrifuge for 10 min at 3000–5000 × *g* using a swing-out rotor. Remove and discard the entire supernatant.

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

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

7. Add 350 µL Buffer BM1, and vortex until the pellet is visibly dissolved.
8. Pipette the sample into a 2 mL processing tube.

   Note: Use the 2 mL processing tubes included in the PAXgene Blood miRNA Kit.
9. Load the open processing tubes containing sample into the QIAcube shaker (see Figure 5, page 45, and Figure 9, page 49). The sample positions are numbered for ease of loading. Insert shaker rack plugs (included with the QIAcube) into the slots at the edge of the shaker rack next to each processing tube. This enables detection of samples during the load check.

   Note: Make sure that the correct shaker adapter (shaker adapter, 2 mL, safe-lock tubes, marked with a "2", included with the QIAcube) is installed.

   Note: If processing fewer than 12 samples, make sure to load the shaker rack as shown in Figure 9, page 49. One or 11 samples cannot be processed. The position numbers in the shaker rack correspond to the position numbers in the centrifuge.
10. Close the QIAcube hood (see Figure 3, page 41).
11. Select the "PAXgene Blood miRNA Part A" protocol and start the protocol. Follow the instructions given on the QIAcube touchscreen.

    Note: Make sure that both program parts (part A and part B) are installed on the QIAcube (see "Installing protocols on the QIAcube instrument", page 42).

    Note: The QIAcube will perform load checks for samples, tips, rotor adapters, and reagent bottles.
12. After the "PAXgene Blood miRNA Part A" protocol is finished, open the QIAcube hood (see Figure 3, page 41). Remove and discard the PAXgene RNA spin columns from the rotor adapters and the empty processing tubes from the shaker.

Note: 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 7, page 47).

13. Close the lids of all 1.5 mL microcentrifuge tubes containing the purified RNA in the rotor adapters (position 3, lid position L3, see Figure 7, page 47). Transfer the 1.5 mL microcentrifuge tubes onto the QIAcube shaker adapter (see Figure 5, page 45, and Figure 9, page 49).
14. Close the QIAcube hood (see Figure 3, page 41).
15. Select the "PAXgene Blood miRNA Part B" protocol and start the protocol.

    Follow the instructions given on the QIAcube touchscreen.

    Note: 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 is essential for maximum efficiency in downstream applications.
16. After the "PAXgene Blood miRNA Part B" program is finished, open the QIAcube hood (see Figure 3, page 41). Immediately place the microcentrifuge tubes 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.

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

17. 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 miRNA Part B").

    See Appendix B, page 36, for information about quantifying RNA. QIAGEN offers a range of optimized, ready-to-use kits for RT-PCR and real-time PCR that can be

used for specific quantification of purified RNA. For details, visit **www.qiagen.com**.

For accurate quantification of RNA by absorbance at 260 nm, we recommend diluting the sample 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 Buffer BR5 and Tris-HCl buffer as in the samples to be measured. Buffer BR5 has high absorbance at 220 nm, which can lead to high background absorbance levels if the spectrophotometer is not properly zeroed.

Note: For quantification in Tris-HCl buffer, use the following relationship: $A_{260} = 1 \Rightarrow 44$ µg/mL. See Appendix B, page 35.

18. Remove the reagent bottle rack from QIAcube worktable (see Figure 5, page 45), and close all bottles with the appropriately labeled lids. Buffer in bottles can be stored at room temperature (15–25°C) for up to 3 months. Remove and discard remaining reagents in the processing tubes in the QIAcube microcentrifuge tube slots (see Figure 5, page 45). Remove and discard rotor adapters from the centrifuge (see Figure 5, page 45). Empty the QIAcube waste drawer (see Figure **3**, page 41). Close the QIAcube hood, and switch off the instrument with the power switch (see Figure 3, page 41).

* 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.

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
Low RNA yield	
a) Less than 2.5 mL blood collected in PAXgene Blood RNA Tube	Ensure that 2.5 mL blood is collected in the PAXgene Blood RNA Tube (see *PAXgene Blood RNA Tube Instructions for Use (Handbook)*).
b) RNA diluted in water for A_{260} measurement	For accurate quantification, RNA must be diluted in 10 mM Tris-HCl, pH 7.5 (see Appendix B, page 35).
c) RNA still bound to PAXgene RNA spin column membrane	Repeat RNA elution, but incubate the PAXgene RNA spin column on the benchtop for 10 min with Buffer BR5 before centrifugation.
d) Cell debris transferred to PAXgene RNA spin column	Be sure to vortex the pellet at step 3 of the manual protocol or step 5 of the automated protocol (however, the presence of small debris does not affect the procedure).
e) Supernatant not completely removed after centrifuging PAXgene Blood RNA Tube	Be sure to remove the entire supernatant. If decanting the supernatant, remove drops from the rim of the tube by dabbing onto a paper towel.

	Comments and suggestions
f) Blood incubated for less than 2 h in PAXgene Blood RNA Tubes	After collecting blood in PAXgene Blood RNA Tubes, be sure that the overall incubation time at room temperature (15–25°C) sums up to at least 2 h.
g) Manual protocol: general yield is good, but miRNA content is low	Ensure that the sample and the isopropanol in step 8 of the manual protocol are mixed completely, until no phase separation is visible.
Low A_{260}/A_{280} value	
a) Water used to dilute RNA for A_{260}/A_{280} measurement	Use 10 mM Tris-HCl, pH 7.5, not RNase-free water, to dilute the sample before measuring purity (see Appendix B, page 35).
b) Spectrophotometer not properly zeroed	To zero the spectrophotometer, use a blank containing the same portion of Buffer BR5 and dilution buffer as in the samples to be measured. Buffer BR5 shows high absorbance at 220 nm, which can lead to high background absorbance levels if the spectrophotometer is not properly zeroed.
RNA degraded	
RNase contamination	Although all PAXgene buffers have been tested and are guaranteed RNase-free, RNases can be introduced during use. Be certain not to introduce any RNases during the PAXgene RNA procedure or later handling. See Appendix A (page 34) for general remarks on handling RNA. Do not put RNA samples into a vacuum dryer or microcentrifuge that has been used in DNA preparation where RNases may have been used.
RNA does not perform well in downstream experiments	

	Comments and suggestions
a) Residual gDNA	In general, the amount of genomic DNA (gDNA) in eluates generated with the PAXgene Blood miRNA Kit is very low as long as the kit is stored and used according to the instructions in this handbook (see "Shipping and Storage" and "Product Use Limitations", as well as "Important points before starting" and "Things to do before starting" in the protocol being used). For downstream applications that are especially sensitive to gDNA contamination, a specialized protocol for the QIAcube Connect and Classic helps reduce gDNA content further and can be downloaded from the QIAcube Connect product page on **www.qiagen.com** and from QIAcube Classic class resource page (**www.qiagen.com/QIAcube-Classic**). Also see "DNA contamination" on page 38.
Instrument malfunction	
QIAcube Connect not operated properly	Read the *QIAcube Connect User Manual*, paying careful attention to the Troubleshooting section. Make sure that the QIAcube Connect is properly maintained, as described in the *QIAcube Connect User Manual*.

Appendix A: General Remarks about Handling RNA

Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even minute amounts are sufficient to destroy 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 purification procedure. To create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and nondisposable 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 may carry bacteria and molds which 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: Storage, Quantification and Determination of Quality of RNA

Storage of RNA

Purified RNA may be stored at –20 or –70°C in Buffer BR5. Under these conditions, no degradation of RNA is detectable after 1 year.

Quantification of miRNA

Since the RNA eluate obtained using this procedure is enriched in various small RNA species, the yield of specific small RNA species (e.g. miRNA) cannot be quantified by OD measurement or fluorogenic assays. Instead, we recommend using quantitative, real-time RTPCR assays, such as the miRCURY® LNA® miRNA Probe PCR system, specific for the type of small RNA under study. For example, to estimate miRNA yield, an assay directed against any miRNA known to be adequately expressed in the samples being processed may be used.

The miRCURY LNA miRNA Probe PCR System is a miRNA-specific, LNA-based system designed for sensitive and accurate detection of miRNA by quantitative, real-time PCR using dual labelled probes. The method is based on universal reverse transcription (RT), followed by real-time PCR amplification with LNA-enhanced primers and probe. See page 52 for Ordering Information.

Quantification of RNA

The concentration of RNA can be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer (see "Spectrophotometric quantification of RNA" below). For small amounts of RNA, however, it may not be possible to accurately determine amounts photometrically. RNA in eluates can be quantified on the QIAxpert® System (**www.qiagen.com/QIAxpert**) or QIAxcel Connect, fluorometric quantification, or quantitative, real-time RT-PCR.

Spectrophotometric 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 ($A_{260} = 1 \Rightarrow 44$ 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 37), 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 Buffer BR5 and Tris-HCl buffer as in the samples to be measured. Buffer BR5 has high absorbance at 220 nm, which can lead to high background absorbance levels if the spectrophotometer is not properly zeroed.

* 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.

An example of the calculation involved in RNA quantification is shown below:

Volume of RNA sample	= 80 µL
Dilution	= 10 µL of RNA sample + 140 µL 10 mM Tris-HCl, pH 7.5 (1/15 dilution)

Measure absorbance of diluted sample in a cuvette (RNase-free).

A_{260}	= 0.3
Concentration of RNA sample	= 44 × A260 × dilution factor
	= 44 × 0.3 × 15
	= 198 µg/mL
Total yield	= concentration × volume of sample in milliliters
	= 198 µg/mL × 0.08 mL
	= 15.8 µg RNA

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 spectrum, 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 1.9–2.1† in 10 mM Tris-HCl, pH 7.5. Always be sure to calibrate the spectrophotometer with the same solution used for dilution.

* 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.

† Values up to 2.3 are routinely obtained for pure RNA (in 10 mM Tris-HCl, pH 7.5) with some spectrophotometers.

DNA contamination

No currently available purification method can guarantee that RNA is completely free of DNA, even when it is not visible on an agarose gel or on capillary electrophoresis instruments. While the vast majority of cellular DNA will be removed by the DNase digestion step, trace amounts may still remain in the purified RNA. For some downstream applications that are especially sensitive to genomic DNA contamination, a specialized protocol for the QIAcube Connect helps reduce genomic DNA content further. This protocol has a longer runtime and could result in overall reduced RNA yields. Download the protocol from the QIAcube Connect product page at **www.qiagen.com** (also see page 12).

For analysis of very low abundance targets, any interference by residual DNA contamination can be detected by performing real-time RT-PCR control experiments in which no reverse transcriptase is added prior to the PCR step.

To prevent any interference by DNA in real-time RT-PCR applications, such as with Rotor-Gene® Q and Applied Biosystems® instruments, we recommend designing primers that anneal at intron splice junctions so that genomic DNA will not be amplified. QuantiNova® LNA PCR Assays and Panels from QIAGEN (www.qiagen.com/GeneGlobe) are designed for analysis of RNA sequences using SYBR® Green detection in real-time RT-PCR without detection of genomic DNA where possible. For real-time RT-PCR assays where amplification of genomic DNA cannot be avoided, the QuantiNova Reverse Transcription Kit provides fast cDNA synthesis with integrated removal of genomic DNA contamination (see Ordering Information, page 52).

Integrity of RNA

The integrity and size distribution of total RNA purified with PAXgene Blood miRNA Kit can be checked by denaturing agarose gel electrophoresis and ethidium bromide staining* or by using the QIAxpert System or QIAxcel Connect System. The respective ribosomal RNAs should appear as sharp bands or peaks. The apparent ratio of 28S rRNA to 18S rRNA should be approximately 2:1. In contrast to other RNA isolation procedures, ribosomal bands or peaks of a specific sample should be sharp and additionally a smear toward smaller sized RNAs should appear. This smear contains small RNA species, such as miRNA.

* 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.

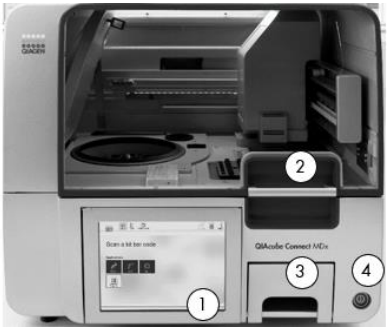
Appendix C: Using the QIAcube Instrument

Ensure that you are familiar with operating the QIAcube instruments (also see page 12). Please read the QIAcube instrument user manuals and any additional information supplied with the QIAcube instrument, paying careful attention to the safety information, before beginning the automated PAXgene Blood miRNA protocol.

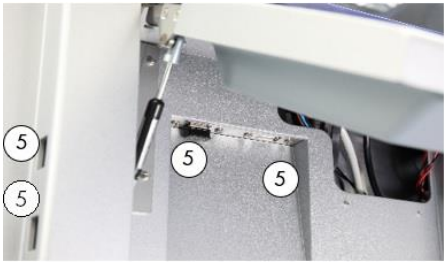
Starting the QIAcube instrument

Close the QIAcube instrument hood, and switch on the QIAcube instrument with the power switch (see Figure 3, page 41).

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



Front view of the QIAcube instrument



Pulled-out touchscreen



Rear view of QIAcube instrument (left side)



Rear view of QIAcube instrument (right side)

Figure 3A. External features of the QIAcube instrument.

1	Touchscreen	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)
2	Hood	6	RJ-45 Ethernet port
3	Waste drawer	7	Power cord socket
4	Front power switch	8	Cooling air outlet

Installing protocols on the QIAcube instrument

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

Protocols are provided at **www.qiagen.com** and need to be downloaded to the USB stick supplied with the QIAcube instrument and transferred to the QIAcube instrument via the USB port. Save the files on the USB stick in a folder named New Protocols.

The USB port, located on the side of the touchscreen (see Figure 3, page 41), allows connection of the QIAcube instrument to the USB stick supplied with the QIAcube instrument. Data files, such as log files or report files, can also be transferred via the USB port from the QIAcube instrument to the USB stick.

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

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

Loading the QIAcube instrument

To save time, loading can be performed during one or both of the 10 min centrifugation steps (steps 3 and 5) in "Protocol: Automated Purification of Total RNA, Including miRNA, from Human Whole Blood Collected into PAXgene Blood RNA Tubes", page 24.

Reagent bottles

Before every run on the QIAcube instrument, carefully fill the 4 reagent bottles listed in Table 1 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 miRNA 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 QIAcube instrument worktable as shown (Figure 4 and Figure 5).

Note: The supplied volume of Buffer BM2 will not fill a reagent bottle to the indicator level. Buffers BM3 and BM4 may not fill the bottle to the indicator level after processing multiple samples in previous runs.

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

Note: Buffer volumes provided in the PAXgene Blood miRNA Kit are sufficient for a maximum of 7 RNA preparation runs on the QIAcube instrument. Multiple runs with few samples should be avoided in order allow sufficient buffer volumes for processing the full 50 samples.

Table 1. Positions in the reagent bottle rack

Position	Reagent
1	Buffer BM2
2	100% isopropanol
3	Buffer BM3*
4	Buffer BM4*
5	– (leave empty)
6	– (leave empty)

* Buffer BM3 and Buffer BM4 are supplied as concentrates. Before using for the first time, add appropriate volumes of ethanol (96–100%, purity grade p.a.) as indicated on the bottle to obtain a working solution.

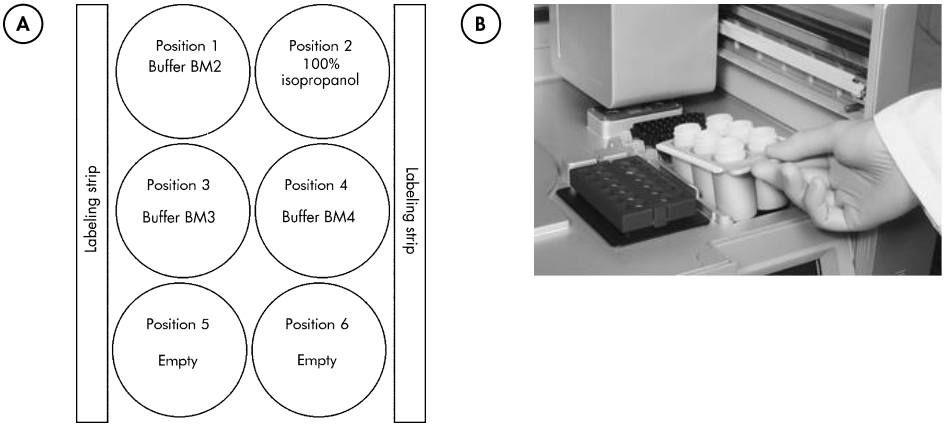


Figure 4. Loading the reagent bottle rack. [A] Schematic of positions and contents of bottles in the reagent bottle rack. [B] Loading the rack onto the QIAcube instrument.

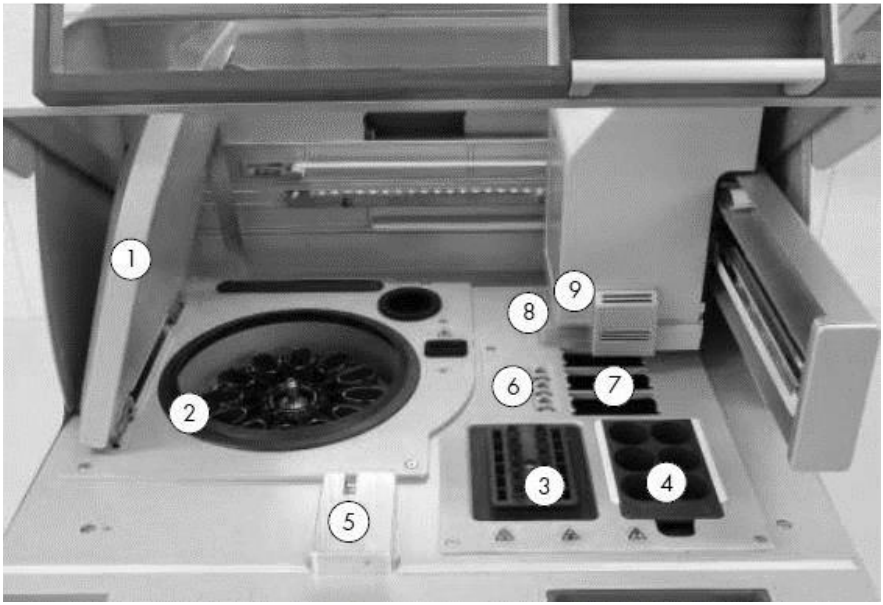


Figure 5. Internal view of the QIAcube instrument.

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

Spin columns, microcentrifuge tubes and QIAcube instrument plasticware

Place 2 tip racks filled with Filter-Tips 1000 µL onto the QIAcube instrument (see Figure **5**, page 45). Refill racks with tips when necessary.

Note: Only use 1000 µL Filter-Tips designed for use with the QIAcube instrument.

Label rotor adapters and microcentrifuge tubes for each sample using a permanent pen. Open the PAXgene Shredder spin columns to be used, and cut the lids off completely using scissors (see Figure 6, page 46).

Note: For proper operation of the QIAcube instrument robotic gripper, completely remove (cut off) the lids and all plastic parts connecting the lid to the PAXgene Shredder spin columns (see Figure 6). Otherwise, the robotic gripper cannot grip the spin columns properly.

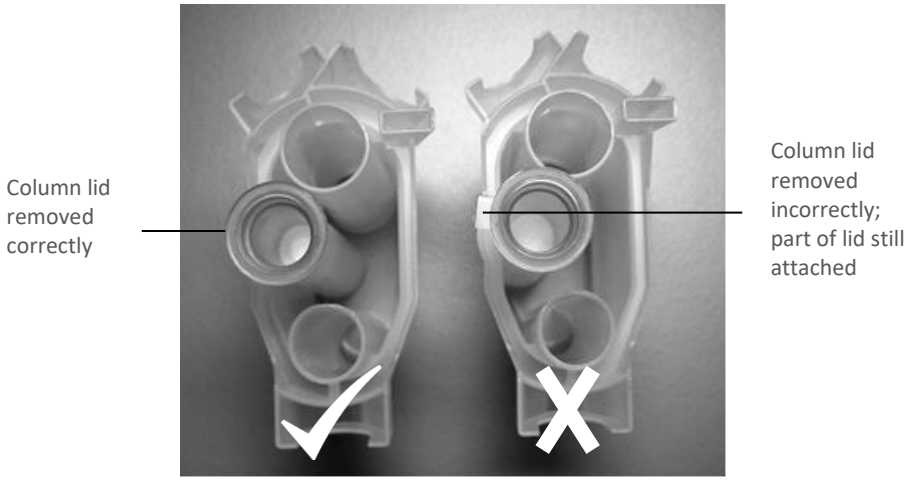


Figure 6. Loading the PAXgene Shredder spin column. The PAXgene Shredder spin column is loaded into the middle position of the rotor adapter. Cut off the lid before loading the column.

Loading a PAXgene Shredder spin column

Load the PAXgene RNA spin column, PAXgene Shredder spin column (without lid, see Figure 6, page 46) and labeled microcentrifuge tube into the appropriate positions in each labeled rotor adapter as shown in Table 2 and Figure 7.

Note: Make sure that the spin column and microcentrifuge tube 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 2. Labware in the rotor adapter

Position	Reagent	Lid position
1	PAXgene RNA spin column (red)	L1
2	PAXgene Shredder spin column (lilac)*	–
3	Microcentrifuge tube†	L3

* Cut off lid before placing in rotor adapter.

† Use the microcentrifuge tubes (1.5 mL) included in the PAXgene Blood miRNA Kit.

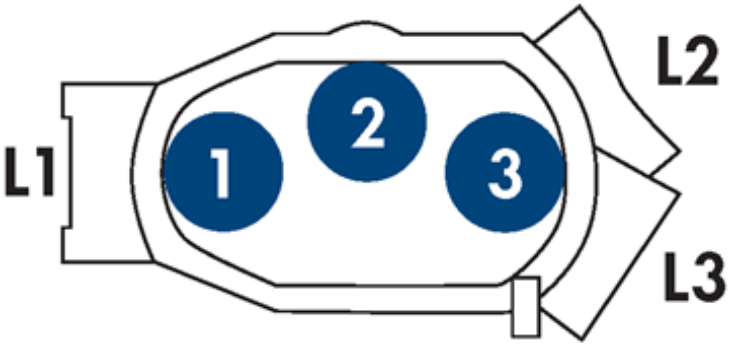


Figure 7. Positions in the rotor adapter. The rotor adapter has three tube positions (1–3) and three lid positions (L1–L3).

Loading the centrifuge

Load the assembled rotor adapters into the centrifuge buckets as shown in Figure 8.

Note: If processing fewer than 12 samples, make sure to load the centrifuge rotor so that it is balanced radially (see Figure 9, page 49). 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 8. Loading the centrifuge. Load the assembled rotor adapters into the centrifuge buckets.

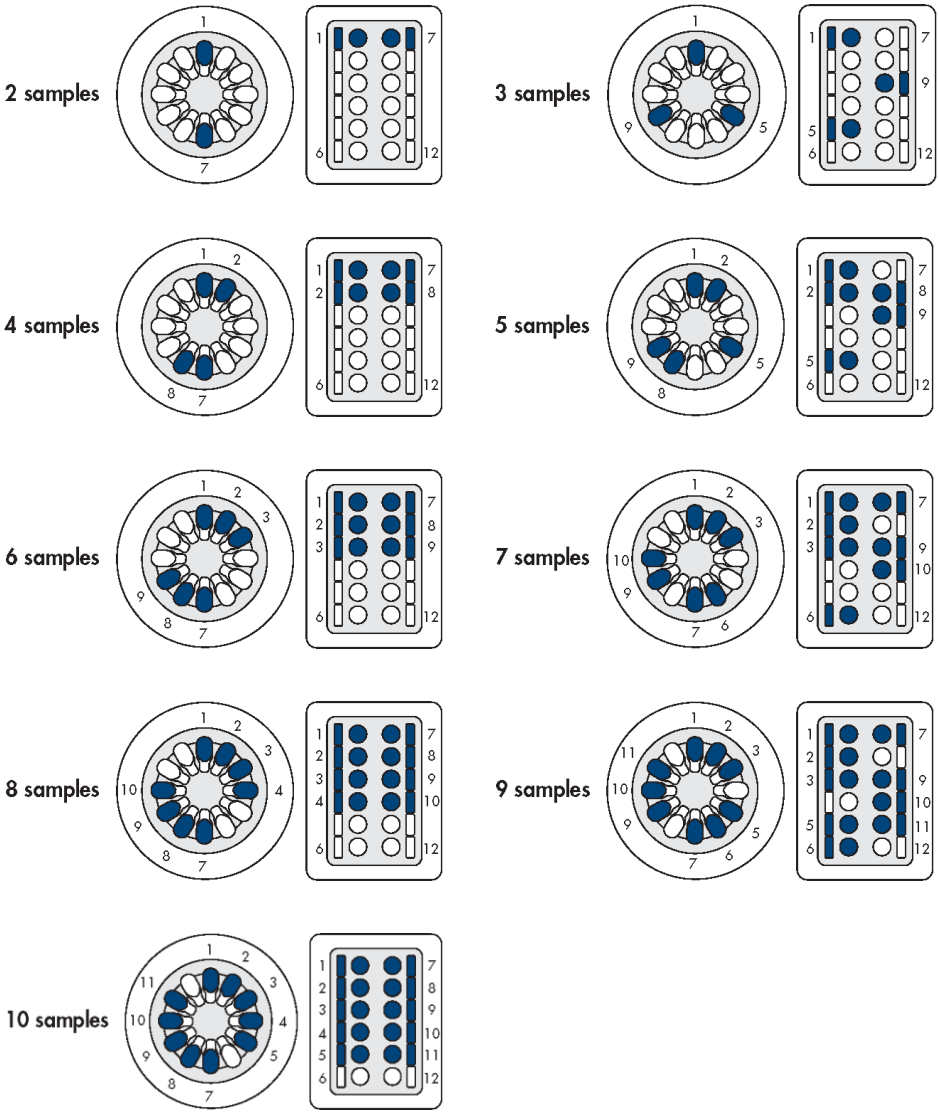


Figure 9. Loading the centrifuge and shaker. Centrifuge and shaker positions are shown for processing from two (2 samples) to ten (10 samples) samples. One or 11 samples cannot be processed.

Processing tubes

Remove any processing tubes left in the microcentrifuge tube slots from previous runs (see Figure 5, page 45). Fill 3 processing tubes with the amount of reagents given in Table 3. Label the tubes clearly with reagent names and place them into the appropriate position in the microcentrifuge tube slots, as indicated in Table 4. Pipet the indicated volume of DNA digestion buffer (RDD) into a processing tube, and add the indicated volume of DNase I stock solution. Mix by gently pipetting the complete mixture up and down 3 times using a 1000 µL pipette tip. Use the 2 mL processing tubes included in the PAXgene Blood miRNA Kit.

Note: Be sure to only pipette the required volume as indicated in Table 3.

Table 3. Volume of reagents required in processing tubes for the microcentrifuge tube 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 4. Microcentrifuge tube 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 processing tubes included in the PAXgene Blood miRNA Kit.

Ordering Information

Product	Contents	Cat. no.
PAXgene Blood miRNA Kit (50)	50 PAXgene Spin Columns, Processing Tubes, RNase-Free DNase I, RNase-Free Reagents and Buffers. To be used in conjunction with the PAXgene Blood RNA Tubes	763134
PAXgene Blood RNA Tubes (100)	100 blood collection tubes	762165
Related products		
QIAcube Connect*	Instrument, connectivity package, 1-year warranty on parts and labor	Inquire
QIAcube Connect MDx	Instrument, 1-year warranty on parts and labor	9003070
Starter Pack, QIAcube	Reagent bottle racks (3); 200 µL Filter-Tips (1024); 1000 µL Filter-Tips (1024); 30 mL reagent bottles (12); 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 the QIAcube	990394
Reagent Bottle Rack	Rack for accommodating 6 × 30 mL reagent bottles on the QIAcube Connect worktable	9026197
Rotor Adapter Holder	Holder for 12 disposable rotor adapters; for use with the QIAcube	990392
QuantiNova Reverse Transcription Kit (50)	For 50 × 20 µL reactions: 100 µL 8x gDNA Removal Mix, 50 µL Reverse Transcription Enzyme, 200 µL Reverse Transcription Mix (containing RT primers), 100 µL Internal Control RNA, 1.9 mL RNase-Free Water	205411

* All QIAcube Connect instruments are provided with a region-specific connectivity package, including tablet and equipment necessary to connect to the local network. Further, QIAGEN offers comprehensive instrument service products, including service agreements, installation, introductory training, and preventive subscription. Contact your local sales representative to learn about your options.

Product	Contents	Cat. no.
QuantiNova Reverse Transcription Kit (200)	For 200 × 20 µL reactions: 4 100 µL 8x gDNA Removal Mix, 4 × 50 µL Reverse Transcription Enzyme, 4 × 200 µL Reverse Transcription Mix (containing RT primers), 4 × 100 µL Internal Control RNA, 4 × 1.9 mL RNase-Free Water	205413
miRCURY LNA miRNA Focus PCR Panels	miRCURY LNA miRNA PCR Panels for application-based miRNome profiling, available in 96-well or 384-well format; for SYBR Green-based detection	339325
miRCURY LNA miRNA miRNome PCR Panels	miRCURY LNA miRNA PCR Panels for PCR-based miRNome profiling, available in 384-well format; for SYBR Green-based detection	339322
miRCURY LNA Probe PCR Kit (200)*	For 200 reactions: 2X QuantiNova Probe Master Mix, 10X miRCURY Probe Univ. Primer, Rox Reference Dye, RNase-Free Water	339371

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
Related products that can be ordered from BD*		
Blood Collection Set	BD Vacutainer® Safety-Lok® 6 Blood Collection Set: 21G, 0.75 inch needle, 12 inch 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
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. To find out more about these accessories, including how to order, visit www.preanalytix.com.

Document Revision History

Date	Changes
04/2020	Revisions throughout document to reflect changes related to automation on QIAcube Connect; general update into new handbook template.
08/2020	Date change, page count update
02/2026	Updated the Kit Contents table. New Data with miRCURY LNA PCR and language adaptation throughout the document. Updated the Ordering Information section. Addition of QIAcube Connect MDx wherever applicable. QIAGEN technical service added in Safety Information; "Total" removed in Quality Control Management System; application modified in introduction and troubleshooting guide; manual protocol centrifuge speed correction; Appendix A adaptation; QIAxcel Connect System added where applicable; updated street address of PreAnalytiX GmbH from "Feldbachstrasse" to "Garstligweg 8". Removal of product warranty.



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 QIAGEN Technical Service or your local distributor.

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