August 2020

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.

PreAnalytiX
A QIAGEN / BD Company

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PreAnalytiX Distributors

PreAnalytiX products are manufactured for PreAnalytiX by QIAGEN or BD and are distributed for PreAnalytiX by QIAGEN or BD. Products cannot be ordered at PreAnalytiX GmbH.

Please see the last page for contact information for your local PreAnalytiX distributor.

Contents

Kit Contents

PAXgene Blood miRNA Kit	**(50)**
Catalog no.	**763134**
Number of preps	**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 × 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
Handbook	1

* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 7 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.

Product Warranty and Satisfaction Guarantee

PreAnalytiX guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, PreAnalytiX will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a PreAnalytiX product does not meet your expectations, simply call your local QIAGEN Technical Service Department or PreAnalytiX distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

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

Quality Control

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

Technical Assistance

At PreAnalytiX and 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 sample and assay technologies and the

use of PreAnalytiX and QIAGEN products. If you have any questions or experience any difficulties regarding the PAXgene Blood miRNA Kit or PreAnalytiX products in general, please do not hesitate to contact us.

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

For technical assistance and more information, please contact Technical Services at **www.preanalytix.com** or call your local distributor (see last page or visit **www.preanalytix.com**).

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.preanalytix.com/resources where you can find, view, and print the SDS for each PreAnalytiX kit and kit component.

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

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.

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
- Microarrays
- 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 50 for Ordering Information).

Principle and procedure

Blood samples are collected in PAXgene Blood RNA Tubes, which contain a proprietary reagent composition based on patented RNA stabilization technology (Please visit www.preanalytix.com/patents). 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. 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 Figures 1 and 2, pages 11 and 13).

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.

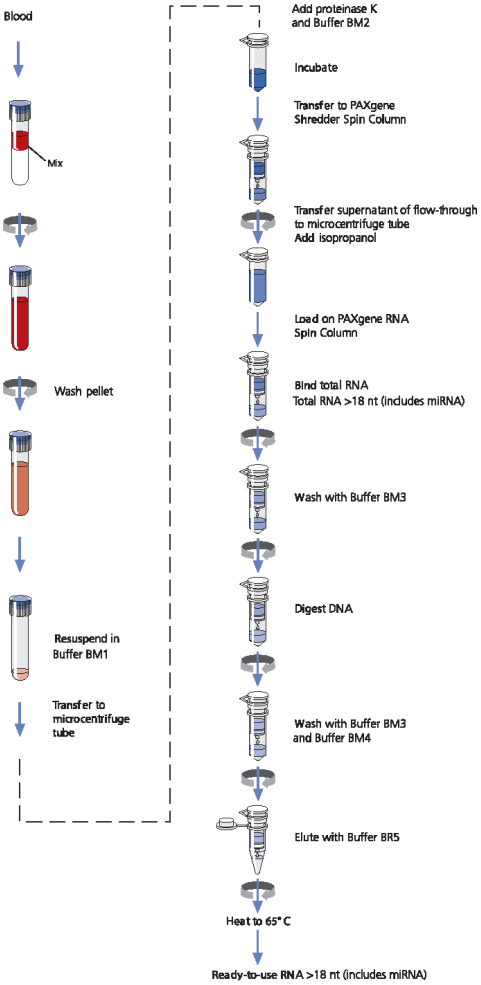


Figure 1. The manual PAXgene Blood miRNA procedure

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

Purification of total RNA, including miRNA, can be fully automated on the QIAcube Connect or the classic 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/qiacubeprotocols**.

QIAcube Connect.

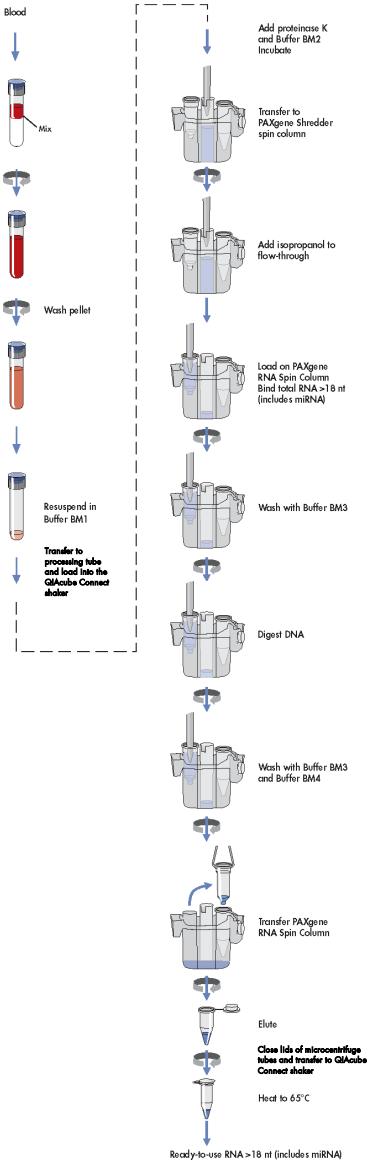


Figure 2. The automated PAXgene Blood miRNA procedure

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 – 4ml)*
- 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

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

For the manual protocol

- Variable speed microcentrifuge* capable of attaining 1000–8000 x *g*, and equipped with a rotor for 2 ml microcentrifuge tubes
- Shaker–incubator† capable of incubating at 55°C and 65°C and shaking at 400 rpm, but not exceeding 1400 rpm (e.g., Eppendorf® Thermomixer Compact* or equivalent)

For the automated protocol

- QIAcube Connect† (QIAGEN, please inquire for cat. no.; also see page 12)
- QIAcube Connect 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 accessories
- Rotor Adapter Holder (QIAGEN, cat. no. 990392) ‡
- Scissors

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

† 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).

Protocol: Manual Purification of Total RNA, Including miRNA, from Human Whole Blood Collected into PAXgene Blood RNA Tubes

Important things 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 pipet 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*.
- After blood collection incubate the PAXgene Blood RNA Tubes for at least 2 h at room temperature 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 incubate it at room temperature for 2 h before starting the procedure.
- Read the safety information on page 7.
- Read the guidelines on handling RNA (Appendix A, page 33).
- Ensure that instruments such as pipets 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 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 33).

Procedure

1. Centrifuge the PAXgene Blood RNA Tube for 10 min at 3000–5000 x *g* using a swing-out 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), in order 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.

* 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).

3. Vortex until the pellet is visibly dissolved, and centrifuge for 10 min at 3000–5000 x *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 x *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 x *g*. Place the spin column in a new 2 ml processing tube and discard the old processing tube containing flow-through.*

* Flow-through contains Buffer BM2 or Buffer BM3 and is therefore not compatible with bleach. See page 7 for safety information.

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

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

* Flow-through contains Buffer BM2 or Buffer BM3 and is therefore not compatible with bleach. See page 7 for safety information.

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 x *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 flow-through and place the PAXgene RNA spin column in a new 2 ml processing tube (supplied). Centrifuge at 8000–20,000 x *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 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 x *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 35, 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 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 pipet 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 7.
- Read the guidelines on handling RNA (Appendix A, page 33).
- Read the *QIAcube Connect User Manual* and any additional information supplied with the QIAcube Connect, paying careful attention to the safety information (also see page 12).
- Ensure that instruments, such as pipets and the QIAcube Connect, 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 33).
- Install the correct shaker adapter (included with the QIAcube Connect; 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 Connect", page 41.

Procedure

1. Close the QIAcube Connect hood, and switch on the QIAcube Connect with the power switch (see Figure 3, page 40).

   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 hood, and load the necessary reagents and plasticware into the QIAcube Connect. See "Loading the QIAcube Connect", page 41.

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

3. Centrifuge the PAXgene Blood RNA Tube for 10 min at 3000–5000 x *g* using a swing-out 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), in order 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.

4. 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.

5. Vortex until the pellet is visibly dissolved, and centrifuge for 10 min at 3000–5000 x *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.

6. Add 350 µl Buffer BM1, and vortex until the pellet is visibly dissolved.

7. Pipet the sample into a 2 ml processing tube.

   Note: Use the 2 ml processing tubes included in the PAXgene Blood miRNA Kit.

8. Load the open processing tubes containing sample into the QIAcube Connect shaker (see Figure 5, page 44, and Figure 9, page 48). The sample positions are numbered for ease of loading. Insert shaker rack plugs (included with the QIAcube Connect) 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 Connect) is installed.

   Note: If processing fewer than 12 samples, make sure to load the shaker rack as shown in Figure 9, page 48. One 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 instrument hood (see Figure 3, page 40).

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

    Follow the instructions given on the QIAcube Connect touchscreen.

    Note: Make sure that both program parts (part A and part B) are installed on the QIAcube Connect instrument (see "Installing protocols on the QIAcube Connect", page 41).

    Note: The QIAcube Connect will perform load checks for samples, tips, rotor adapters, and reagent bottles.

11. After the "PAXgene Blood miRNA Part A" protocol is finished, open the QIAcube Connect instrument hood (see Figure 3, page 40). 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 46).

12. 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 46). Transfer the 1.5 ml microcentrifuge tubes onto the QIAcube Connect shaker adapter (see Figure 5, page 44, and Figure 9, page 48).

13. Close the QIAcube Connect instrument hood (see Figure 3, page 40).
14. Select the "PAXgene Blood miRNA Part B" protocol, and start the protocol.

    Follow the instructions given on the QIAcube Connect 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.
15. After the "PAXgene Blood miRNA Part B" program is finished, open the QIAcube Connect instrument hood (see Figure 3, page 40). 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 Connect. 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 miRNA Part B").

    See Appendix B, page 35, 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 relationship

A_{260} = 1 ⇒ 44 µg/ml. See Appendix B, page 35.

17. Remove the reagent bottle rack from QIAcube Connect worktable (see Figure 5, page 44), 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 Connect microcentrifuge tube slots (see Figure 5, page 44). Remove and discard rotor adapters from the centrifuge (see Figure 5, page 44). Empty the QIAcube Connect waste drawer (see Figure 3, page 40). Close the QIAcube Connect instrument hood, and switch off the instrument with the power switch (see Figure 3, page 40).

* 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 also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see last page or visit www.preanalytix.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 to incubate for at least 2 h at room temperature (15–25°C).
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		
a)	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 33) 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.

	Comments and suggestions
RNA does not perform well in downstream experiments	
a) High levels of globin mRNA affect microarray analysis	High levels of globin mRNA in purified RNA from whole blood reduce the sensitivity of gene expression analysis with Affymetrix® GeneChip® arrays. To avoid this problem, we recommend preparing targets for GeneChip arrays using the Ovation® Whole Blood Solution from Tecan (**www.nugen.com**).
b) 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" on page 5, 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 helps reduce gDNA content further and can be downloaded from the QIAcube Connect product page at **www.qiagen.com**. 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. In order 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.

Disposable plasticware

The use of sterile, disposable polypropylene tubes is recommended throughout the procedure. These tubes are generally RNase-free and do not require pretreatment to inactivate RNases.

Nondisposable plasticware

Nondisposable plasticware should be treated before use to ensure that it is RNase- free. Plasticware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA* followed by RNase-free water (see "Solutions", page 34). Alternatively, chloroform- resistant plasticware can be rinsed with chloroform* to inactivate RNases.

Glassware

Glassware should be treated before use to ensure that it is RNase-free. Glassware used for RNA work should be cleaned with a detergent,* thoroughly rinsed, and oven baked at 240°C for at least 4 hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with DEPC* (diethyl pyrocarbonate). Fill glassware with 0.1% DEPC (0.1% in water), allow to stand overnight (12 h) at 37°C, and then autoclave or heat to 100°C for 15 minutes to eliminate residual DEPC.

Electrophoresis tanks

Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS),* thoroughly rinsed with RNase-free water, and then rinsed with ethanol† and allowed to dry.

Solutions

Solutions (water and other solutions) should be treated with 0.1% DEPC. DEPC is a strong, but not absolute, inhibitor of RNases. It is commonly used at a concentration of 0.1% to inactivate RNases on glass or plasticware or to create RNase-free solutions and water. DEPC inactivates RNases by covalent modification. Add 0.1 ml DEPC to 100 ml of the solution to be treated and shake vigorously to bring the DEPC into solution. Let the solution incubate for 12 hours at 37°C. Autoclave for 15 minutes to remove any trace of DEPC. DEPC will react

* 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.

† Plastics used for some electrophoresis tanks are not resistant to ethanol. Take proper care and check the supplier's instructions.

with primary amines and cannot be used directly to treat Tris* buffers. DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and CO2. When preparing Tris buffers, treat water with DEPC first, and then dissolve Tris to make the appropriate buffer. Trace amounts of DEPC will modify purine residues in RNA by carbethoxylation. Carbethoxylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA:RNA or RNA:RNA hybrids is not seriously affected unless a large fraction of the purine residues have been modified. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

Note: PAXgene RNA buffers are guaranteed RNase-free without using DEPC treatment and are therefore free of any DEPC contamination.

Appendix B: Storage, Quantification and Determination of Quality of RNA

Storage of RNA

Purified RNA may be stored at –20°C 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 RT-PCR assays, such as the miScript® 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 miScript PCR System is a three-component system that covers all the steps of conversion of miRNA and mRNA into cDNA and detection of miRNAs in SYBR® Green real-time PCR. A single cDNA synthesis reaction is sufficient for analysis of multiple miRNAs. The miScript PCR System can also be used for detection of other small RNAs, such as snoRNAs or piRNAs. See page 50 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 Agilent® 2100 Bioanalyzer, 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 ⇒ 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

* 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.

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 = 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 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

Purity of RNA

The ratio of the readings at 260 nm 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}

* 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.

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.

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. 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 50).

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

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 Agilent 2100 Bioanalyzer. 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 towards smaller sized RNAs should appear. This smear contains small RNA species, such as miRNA.

Appendix C: Using the QIAcube Connect

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

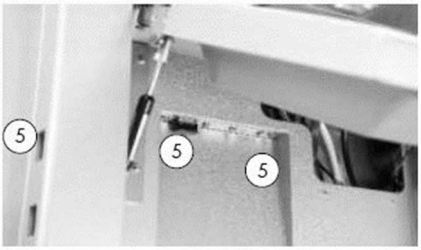
Starting the QIAcube Connect

Close the QIAcube Connect instrument hood, and switch on the QIAcube Connect with the power switch (see Figure 3, page 40).

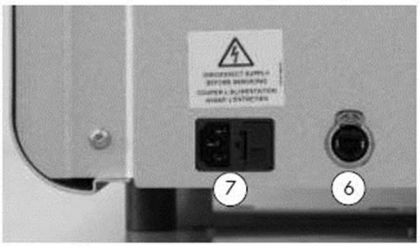
A beeper sounds and the startup screen appears. The instrument automatically performs initialization tests.

* 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.

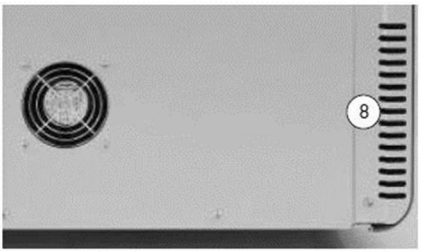
Front view of the QIAcube Connect



Pulled-out touchscreen



Rear view of QIAcube Connect



Rear view of QIAcube Connect

Figure 3. External features of the QIAcube Connect.

1. Touchscreen
2. Hood
3. Waste drawer
4. Front 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

Installing protocols on the QIAcube Connect

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

Protocols are provided at **www.qiagen.com/MyQIAcubeConnect** and need to be downloaded to the USB stick supplied with the QIAcube Connect and transferred to the QIAcube Connect 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 40), allows connection of the QIAcube Connect to the USB stick supplied with the QIAcube Connect. Data files, such as log files or report files can also be transferred via the USB port from the QIAcube Connect 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 Connect

To save time, loading can be performed during one or both of the 10-minute 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 23.

Reagent bottles

Before every run on the QIAcube Connect, 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 Connect worktable as shown (Figure 4 and Figure 5, page 44).

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 Connect. 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.

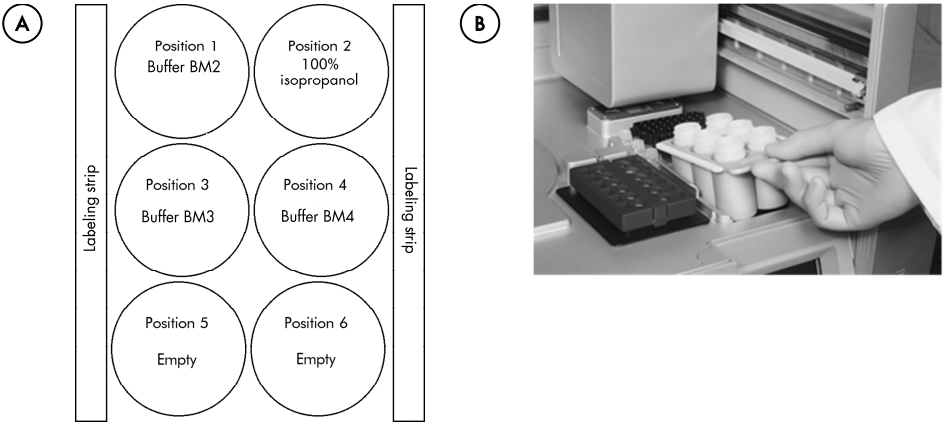


Figure 4. Loading the reagent bottle rack. [**A**] Schematic of positions and contents of bottles in the reagent bottle rack. [**B**] Loading the rack onto the QIAcube Connect.

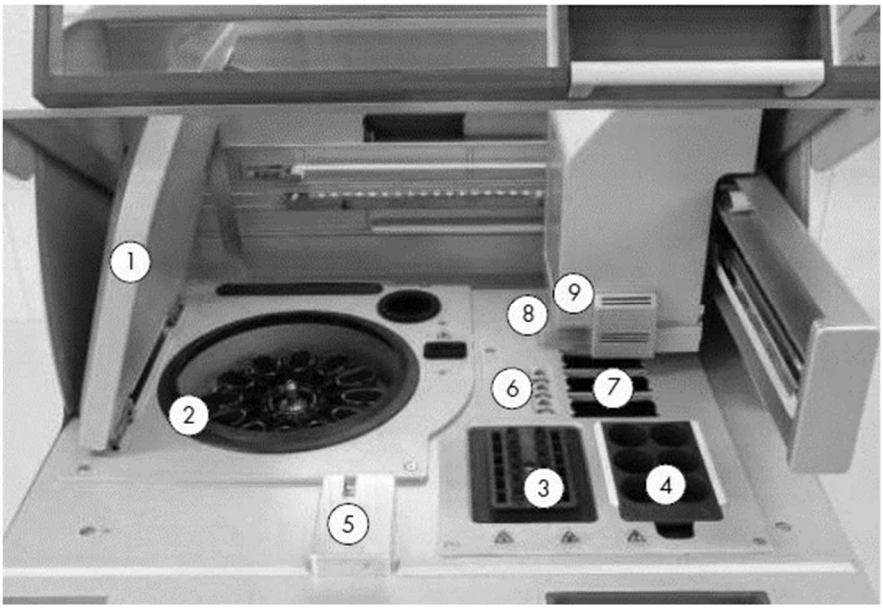


Figure 5. Internal view of the QIAcube Connect.

1. Centrifuge lid
2. Centrifuge
3. Shaker
4. Reagent bottle rack
5. Tip sensor and hood lock
6. Microcentrifuge tube 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, microcentrifuge tubes, and QIAcube Connect plasticware

Place 2 tip racks filled with Filter-Tips 1000 µl onto the QIAcube Connect (see Figure 5, page 44). Refill racks with tips when necessary.

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

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 45).

Note: For proper operation of the QIAcube Connect 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.

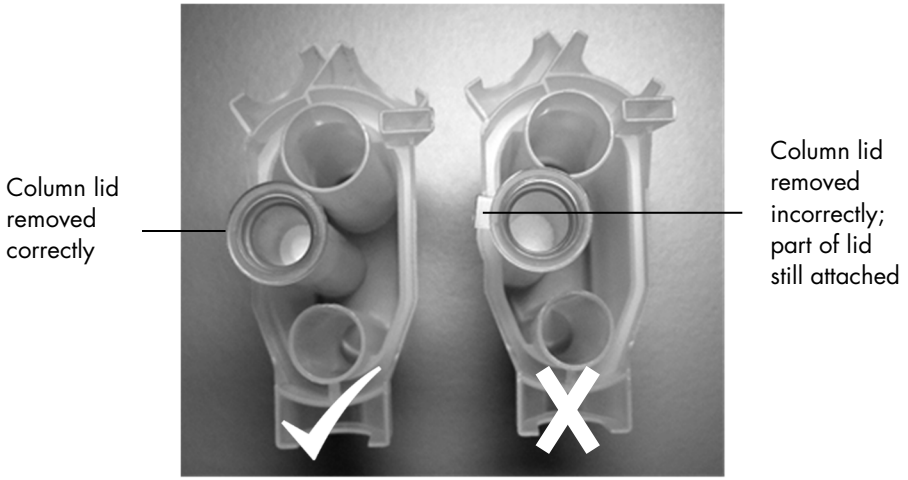


Figure 6. Loading the PAXgene Shredder spin column. The PAXgene Shredder spin column is loaded into the middle position of the rotor adapter. Cut off the lid before loading the column.

Loading a PAXgene Shredder spin column

Load the PAXgene RNA spin column, PAXgene Shredder spin column (without lid, see Figure 6, page 45), 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.

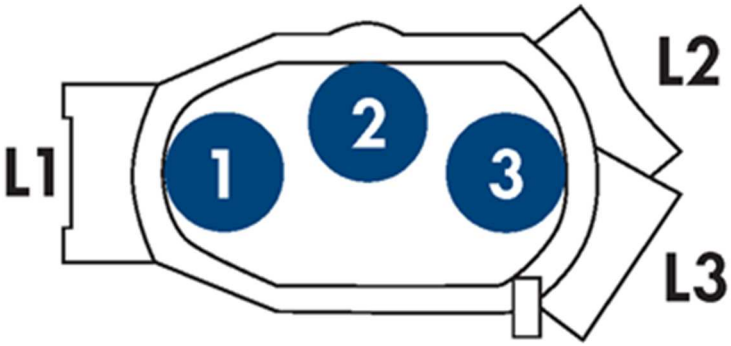


Figure 7. Positions in the rotor adapter. The rotor adapter has three tube positions (1–3) and three lid positions (L1–L3).

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 48). 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.

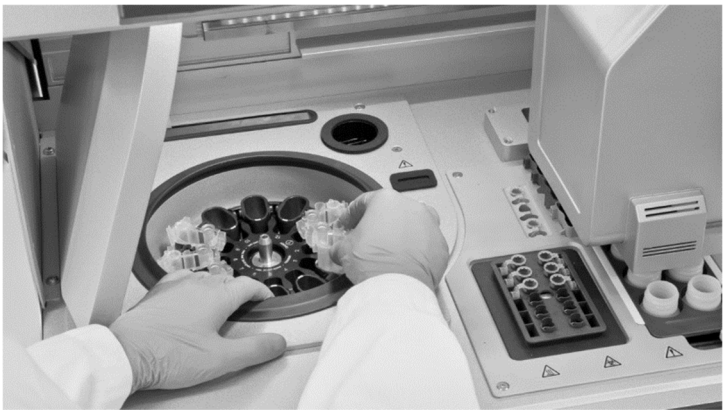


Figure 8. Loading the centrifuge. Load the assembled rotor adapters into the centrifuge buckets.

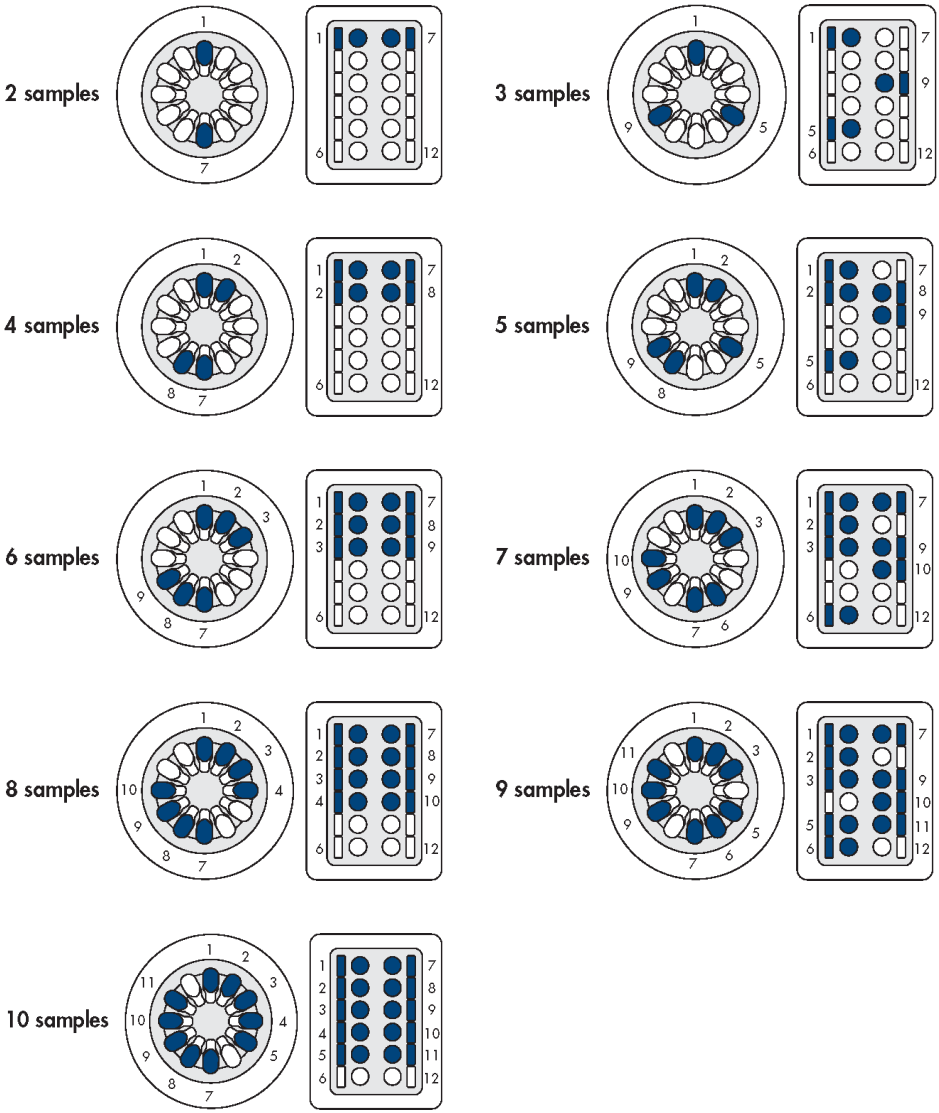


Figure 9. Loading the centrifuge and shaker. Centrifuge and shaker positions are shown for processing from two (2 samples) to ten (10 samples) samples. One or 11 samples cannot be processed.

Processing tubes

Remove any processing tubes left in the microcentrifuge tube slots from previous runs (see Figure 5, page 44). 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 pipet tip. Use the 2 ml processing tubes included in the PAXgene Blood miRNA Kit.

Note: Be sure to only pipet 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
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 Connect reagent bottle rack	990393
Rotor Adapters (10 x 24)	For 240 preps: 240 Disposable Rotor Adapters; for use with the QIAcube Connect	990394

* 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.

Reagent Bottle Rack	Rack for accommodating 6 x 30 ml reagent bottles on the QIAcube Connect worktable	990390
Rotor Adapter Holder	Holder for 12 disposable rotor adapters; for use with the QIAcube Connect	990392
QuantiNova Reverse Transcription Kit (50)	For 50 x 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
QuantiNova Reverse Transcription Kit (200)	For 200 x 20 µl reactions: 4 x 100 µl 8x gDNA Removal Mix, 4 x 50 µl Reverse Transcription Enzyme, 4 x 200 µl Reverse Transcription Mix (containing RT primers), 4 x 100 µl Internal Control RNA, 4 x 1.9 ml RNase-Free Water	205413
miScript II RT Kit (12)*	For 12 cDNA synthesis reactions: miScript Reverse Transcriptase Mix, 10x miScript Nucleics Mix, 5x miScript HiSpec Buffer, 5x miScript HiFlex Buffer, RNase-Free Water	218060
miScript Primer Assay (100)	miRNA-specific primer; available via GeneGlobe	Varies

* Larger kit sizes available; please inquire.

Pathway-Focused miScript miRNA PCR Assay	Array of miRNA assays for a pathway, disease or gene family for various species; available in 96-well, 384-well or Rotor-Disc 100 format	Varies
miRNome miScript miRNA PCR Array	Array of miRNA assays for miRNomes of various species; available in 96-well, 384-well or Rotor-Disc 100 format	Varies
miScript SYBR Green PCR Kit (200)*	For 200 reactions: QuantiTect SYBR Green PCR Master Mix, miScript Universal Primer	218073
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 x 75 mm 4.0 ml draw with Red BD Hemogard closure and paper label; 100/box, 1000/case	368975

* Larger kit sizes available; please inquire.

† 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**.

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.

Document Revision History

Document/ Changes	Date
HB-0198-004: Revisions throughout document to reflect changes related to automation on QIAcube Connect; general update into new handbook template.	May 2020
HB-0198-005: Date change, page count update	August 2020

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PreAnalytiX Worldwide

PreAnalytiX products are distributed by QIAGEN and BD companies

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