QIAsymphony® PAXgene® Blood RNA Kit Handbook

For purification of cellular RNA from whole blood using the QIAsymphony SP instrument.

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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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 page 31 for contact information for your local PreAnalytiX distributor.

Contents

Intended Use

For Research Use Only. Not for use in diagnostic procedures. The performance characteristics of this product have not been fully established.

When the QIAsymphony PAXgene Blood RNA Kit is used in conjunction with the PAXgene Blood RNA Tube, the System provides purified intracellular RNA, including miRNA, for research tests including but not limited to RT-PCR.

The QIAsymphony PAXgene Blood RNA Kit is intended for purification of intracellular RNA, including miRNA, from human whole blood (4.8×10^6–1.1×10^7 leukocytes/ml).

The QIAsymphony PAXgene Blood RNA Kit is not for the isolation of genomic DNA or viral nucleic acids from human whole blood.

All due care and attention should be exercised in the handling of the products.

Summary and Explanation

QIAsymphony technology combines the speed and efficiency of silica-based nucleic acid purification with the convenient handling of magnetic particles (Figure 1). The purification procedure is designed to ensure safe and reproducible handling of potentially infectious samples.

The QIAsymphony PAXgene Blood RNA Kit allows automated, standardized purification on the QIAsymphony SP of total RNA, including miRNA, from 2.5 ml human whole blood collected into PAXgene Blood RNA Tubes. Proven MagAttract® magnetic-particle technology provides high-quality RNA, which is suitable for direct use in downstream applications. The QIAsymphony SP performs all steps of the sample preparation procedure. Up to 72 samples, in 3 batches of 24, can be processed in a single run.

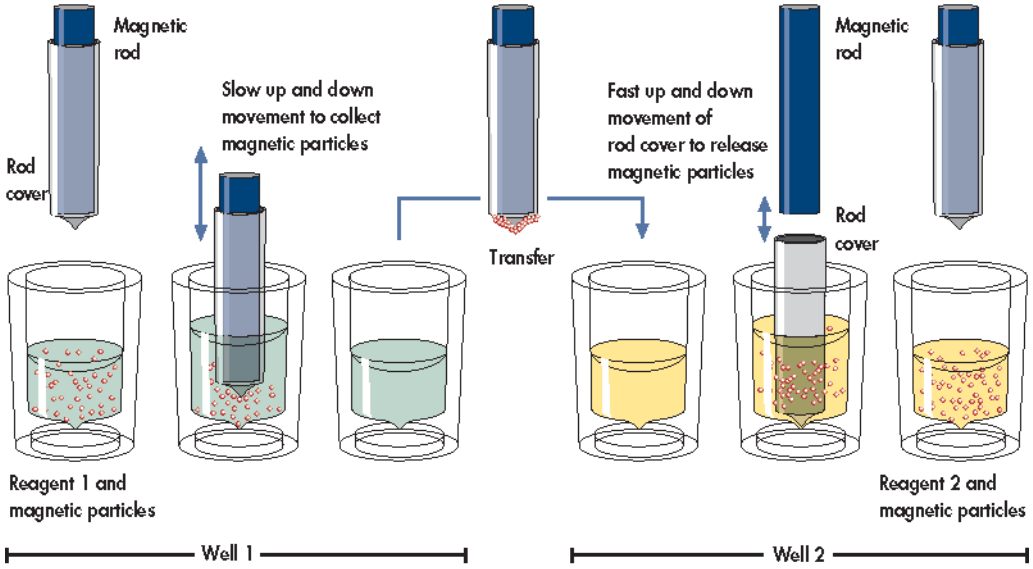

Figure 1. Schematic of the QIAsymphony SP principle. The QIAsymphony SP processes a sample containing magnetic particles as follows. A magnetic rod protected by a rod cover enters a well containing the sample and attracts the magnetic particles. The magnetic rod cover is positioned above another well and the magnetic particles are released. The QIAsymphony SP uses a magnetic head containing an array of 24 magnetic rods, and can therefore process up to 24 samples simultaneously. Steps 1 and 2 are repeated several times during sample processing.

Principle of the Procedure

The simple procedure begins with a centrifugation step to pellet the contents of each PAXgene Blood RNA Tube. After decanting the supernatant, the pellets are then resuspended by vortexing in optimized buffers, which are supplemented with proteinase K. The tubes are transferred to the QIAsymphony SP.

After a precleaning step, RNA binds to the silica surface of MagAttract magnetic particles in the presence of a chaotropic salt and isopropanol. Two prewash steps guarantee that DNase I digestion is optimized. Remaining proteins and contaminants are removed by an additional proteinase K treatment and 3 wash steps, and RNA is eluted in Buffer BR5. Finally, a heat treatment of the eluted RNA provides ready-to-use RNA and ensures consistent performance in downstream applications.

Guaranteed yields of RNA isolated from 2.5 ml healthy, human, whole blood (4.8×10^6– 1.1×10^7 leukocytes/ml) are >3 µg for >95% of the samples processed. Since yields are highly donor-dependent, individual yields may vary. Average yields can be expected in the range between 8 and 16 µg. Purified RNA can be used in downstream applications including but not limited to RT-PCR.

Kit Contents

QIAsymphony PAXgene Blood RNA Kit	**(96)**
Catalog no.	**762635**
Number of preps	**96**
Buffer BR2	25 ml
Buffer BR5	30 ml
Buffer QSX2	30 ml
Buffer RDD	30 ml
Proteinase K	2 x 1.4 ml
DNase I (1500 Kunitz units)*	4 glass vials
RNase-Free Water	2 x 2 ml
Reagent Cartridge†	2
Piercing Lid	2
Reuse Seal Set	2
Secondary Hemogard Closures	2 x 50
Elution Microtubes CL, racked‡	2
Caps for Elution Microtubes‡	55 x 8
Handbook	1

* Kunitz units are the commonly used units for measuring DNase I; see page 14 for definition.

† Prefilled reagent cartridges include buffers that contain a guanidine salt, ethanol, isopropanol, and/or proteinase K. See page 9 for safety information.

‡ Also available separately. See page 28 for ordering information.

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.

Consumables

- 8-Rod Covers (cat. no. 997004)
- Sample Prep Cartridges, 8-well (cat. no. 997002)
- Filter-Tips, 1500 µl (cat. no. 997024)
- Filter-Tips, 200 µl (cat. no. 990332)
- Sterile, aerosol-barrier, RNase-free pipet tips*
- RNase-free syringe and needle (for resuspension of DNase I)
- Crushed ice

Reagents

- PAXgene Blood RNA Tubes (cat. no. 762165; available from BD and BD authorized distributors; see **www.PreAnalytiX.com**)

Equipment

- QIAsymphony SP (cat. no. 9001297)
- Optional: QIAsymphony Cabinet SP (cat. no. 9020244)
- Pipets† (200 µl – 1 ml)
- Centrifuge† capable of attaining 3000–5000 x *g* and equipped with a swing-out rotor and buckets to hold PAXgene Blood RNA Tubes
- Vortex mixer†
- Optional: A step dispenser†, such as the Multipette plus from Eppendorf®, is recommended for optimal processing‡
- Optional: A multitube vortexer†, such as the VX2500 from VWR®, is recommended for optimal processing‡

* Ensure that you are familiar with the guidelines on handling RNA (Appendix A, page 23).

† Ensure that instruments have been checked 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.

Elution in Elution Microtubes CL

Equipment

- PAXgene 96 Incubator Block (cat. no. 9238279)
- Incubator capable of 80°C*
- Spatula to remove the lower plate from the elution microtube rack
- Heavy plate to prevent caps from opening during incubation at 80°C

Elution in 2 ml Sarstedt® tubes

Consumables

- Micro tube 2ml, PP (Sarstedt, cat. no. 72.608) with Screw cap, neutral (Sarstedt, cat. no. 65.716.725)†

Equipment

- Shaker–incubator, heating block, or water bath* capable of incubating at 65°C

For other possible elution formats see **www.qiagen.com/products/qiasymphonysp.aspx** under user support.

* Ensure that instruments have been checked 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.

Warnings and Precautions

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.

The bottles and the troughs of the reagent cartridges contain Buffer BR2 and Buffer QSB1 which contain guanidine thiocyanate, and Buffer QSW5, which contains guanidine hydrochloride. Guanidine thiocyanate and guanidine hydrochloride 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.

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

Reagent Storage and Handling

Except for the DNase I vials, the remaining components of the QIAsymphony PAXgene Blood RNA Kit can be stored at room temperature (15–25°C). Do not store reagent cartridges at temperatures below 15°C.

The QIAsymphony PAXgene Blood RNA Kit contains a ready-to-use proteinase K solution in the enzyme rack of the reagent cartridge (see Figure 2, page 11) and as separate tubes. Proteinase K can be stored at room temperature (15–25°C). To store for extended periods of time, we suggest keeping the enzyme rack with proteinase K and DNase I tubes at 2–8°C.

The DNase I vials are shipped at room temperature. The vials should be stored immediately upon receipt at 2–8°C. When stored at 2–8°C and handled correctly, the lyophilized enzyme can be kept for at least 9 months without showing any reduction in performance. The DNase I stock solution in the enzyme rack has to be stored at 2–8°C (see "Things to do before starting", page 14, for preparation of DNase I stock solution).

For other possible elution formats see **www.qiagen.com/products/qiasymphonysp.aspx** under user support.

Partially used reagent cartridges can be stored for a maximum of 2 weeks, enabling cost-efficient reuse of reagents and more flexible sample processing. If a reagent cartridge is partially used, seal the piercing lid with the Reuse Seal Set provided. To avoid evaporation, seal the reagent cartridge immediately after the end of the protocol run. The reagent cartridge is designed to allow 4 uses with a total opening time of up to 15 hours at a maximum environmental temperature of 30°C.

Specimen Handling and Storage

Blood must be collected in PAXgene Blood RNA Tubes (cat. no. 762165). See the *PAXgene Blood RNA Tube Product Circular* for information about specimen collection and handling.

After collection of the blood sample, it is important to incubate the PAXgene Blood RNA Tube for at least 2 hours at room temperature (15–25°C) before RNA purification. Incubation of the PAXgene Blood RNA Tube overnight may increase RNA yields in some cases. If the blood samples in the PAXgene Blood RNA Tubes were frozen, thaw at room temperature for at least 2 hours before RNA purification.

Procedure

Automated purification on the QIAsymphony SP

The QIAsymphony SP makes automated sample preparation easy and convenient. Samples, reagents and consumables, and eluates are separated in different drawers. Simply load samples, reagents provided in special cartridges, and preracked consumables in the appropriate drawer. Start the protocol and remove purified RNA from the eluate drawer after sample processing is completed. Refer to the *QIAsymphony SP/AS User Manual — Operating the QIAsymphony SP* for operating instructions.

Even though the QIAsymphony SP was designed as a benchtop instrument, the use of the QIAsymphony Cabinet SP will enhance the convenience of using this robotic system. The QIAsymphony Cabinet SP is specifically designed for correct positioning of the QIAsymphony SP instrument. The QIAsymphony Cabinet SP contains a waste compartment, into which used tips from the worktable can be ejected.

“Reagents and Consumables” drawer

Reagent cartridges

Reagents for purification of RNA from PAXgene Blood RNA Tubes are contained in an innovative reagent cartridge (Figure 2). Only buffer RDD has to be loaded in a separate bottle holder position. Each trough of the reagent cartridge contains a particular reagent, such as magnetic particles, enzyme buffer, binding buffer, wash buffer, or elution buffer. Partially used reagent cartridges can be reclosed with Reuse Seal Strips for later use. This avoids generating waste due to leftover reagents at the end of the purification procedure.

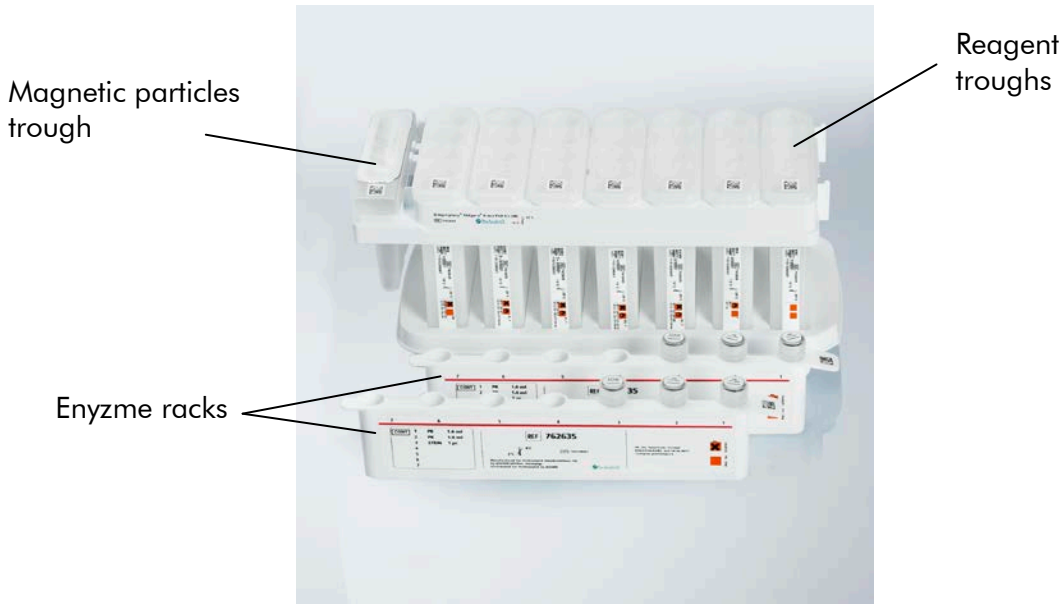

Figure 2. The reagent cartridge contains all reagents required for the protocol run. The cartridge is placed inside the Reagent cartridge holder (not included in image).

Note: Before using a reagent cartridge for the first time, remove the seal from the trough containing the magnetic particles, and replace it with the trough cover. Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex the sealed or covered trough containing the magnetic particles vigorously for at least 3 minutes before first use. Place the reagent cartridge into the reagent cartridge holder. If required by the protocol, place the enzyme rack into the reagent cartridge holder. Place the piercing lid on top of the reagent cartridge (Figure 3). Scan bar code of the buffer RDD bottle and load it in the buffer bottle position.

Important: The piercing lid is sharp; use caution when placing it onto the reagent cartridge. Make sure to place the piercing lid onto the reagent cartridge in the correct orientation.

The reagent cartridge is then loaded into the “Reagents and Consumables” drawer.

Figure 3. Easy worktable setup with reagent cartridges. The reagent cartridge with the piercing lid positioned in the reagent cartridge holder.

Loading plasticware

Sample prep cartridges, 8-Rod Covers (both preracked in unit boxes), and disposable filter-tips (200 µl tips in blue racks, 1500 µl tips in gray racks) are loaded into the “Reagents and Consumables” drawer (see Table 1, page 13).

See Table 1 (page 13) for the consumables required. For plasticware ordering information, see page 28.

Note: Both types of tips have filters to prevent cross-contamination.

Tip rack slots on the QIAsymphony worktable can be filled with either type of tip rack. The QIAsymphony SP will automatically identify the type of tips loaded during the inventory scan.

Note: Do not refill tip racks before starting another protocol run. The QIAsymphony SP can use partially used tip racks.

“Waste” drawer

Sample prep cartridges and 8-Rod Covers used during a run are re-racked in empty unit boxes in the “Waste” drawer. Ensure that the “Waste” drawer contains sufficient empty unit boxes for plastic waste generated during the protocol run.

Note: Ensure that the covers of the unit boxes are removed before loading the unit boxes into the “Waste” drawer. If you are using 8-Rod Cover boxes for collecting used sample prep cartridges and 8-Rod Covers, ensure that the box spacer has been removed.

A bag for used filter-tips must be attached to the front side of the “Waste” drawer. If a QIAsymphony Cabinet SP is used, the waste compartment, into

which used tips from the worktable are ejected, must be checked for sufficient space to collect waste tips.

Note: The presence of a tip disposal bag is not checked by the system. Ensure that the tip disposal bag is properly attached before starting a protocol run (see the *QIAsymphony SP/AS User Manual — Operating the QIAsymphony SP*).

A waste container collects all liquid waste generated during the purification procedure. The "Waste" drawer can only be closed if the waste container is in place. Furthermore, a liquid-level sensor detects the level of liquid in the waste container. The system notifies the user if there is not enough capacity left in the container for liquid waste from the queued batch.

"Eluate" drawer

The required elution rack is loaded into the "Eluate" drawer. Do not load a 96 well plate into "Elution slot 4". Use "Elution slot 1" with the corresponding cooling adaptor so that eluates will be cooled at the end of the run.

Inventory scan

Before starting a run, the instrument checks that sufficient consumables for the queued batch(es) have been loaded into the corresponding drawers (Table 1).

Table 1. Consumables required for purification of cellular RNA from whole blood

Number of samples	24	48	96
Reagent cartridges	1	1	2
Sample prep cartridges*	27	54	81
8-Rod Covers†	3	6	9
1500 µl tips‡ (tip racks)	132 (5)	264 (9)	396 (13)
200 µl tips‡ (tip racks)	28 (1)	56 (2)	84 (3)

* 28 sample prep cartridges/unit box.

† Twelve 8-Rod Covers/unit box.

‡ 32 tips/tip rack; depending on the reagent cartridge status, the inventory scan requires additional tips (two 200 µl and nine 1500 µl tips).

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

Important points before starting

- Blood must be collected in PAXgene Blood RNA Tubes (cat. no. 762165).
- Use of a step dispenser, such as the Multipette plus from Eppendorf, and a multitube vortexer, such as the VX2500 from VWR, is recommended.
- All steps of the QIAsymphony PAXgene Blood RNA protocol for purification of total RNA, including miRNA, should be performed at 15–25°C.

Things to do before starting

- After collection of the blood sample, it is important to incubate the PAXgene Blood RNA Tube for at least 2 hours at room temperature (15–25°C) before RNA purification. Incubation of the PAXgene Blood RNA Tube overnight may increase RNA yields in some cases. If the blood samples in the PAXgene Blood RNA Tubes were frozen, they must be thawed at room temperature for at least 2 hours before RNA purification.
- Prepare DNase I stock solution before using the reagent cartridge for the first time. Dissolve 2 vials of the lyophilized DNase I (1500 Kunitz units* each) in 900 µl per vial of the RNase-free water provided. To avoid loss of DNase I, do not open the vial. Instead, inject RNase-free water into each vial using an RNase-free needle and syringe. Mix gently by inverting the vial. Do not vortex. Transfer the contents of both vials (1.8 ml total) to the tube at position 3 of the enzyme rack on the reagent cartridge.
- DNase solution can be stored at 2–8°C for up to 6 weeks.
- Buffer BR2 (reagent trough 1 and separate bottle) may form a precipitate upon storage. If a precipitate forms, warm to 37°C to redissolve.
- Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex the sealed or covered trough containing the magnetic particles vigorously for at least 3 minutes before first use.
- If using a new reagent cartridge, remove the seal from the trough containing the magnetic particle. Make sure that the piercing lid is placed on the reagent cartridge.

* 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).

- If using a previously used reagent cartridge, make sure that the Reuse Seal Strips and rough cover have been removed.
- The enzyme rack must be attached to the reagent cartridge, and the tube caps must be removed.
- Orient sample racks so that the bar codes face the bar code reader on the left side of the QIAsymphony SP.
- We recommend using Elution Microtubes CL, racked (provided) for elution. Alternatively, 2 ml Sarstedt tubes can be used (see page 8). For other possible elution formats, see **www.qiagen.com/products/qiasymphonysp.aspx** under Product Resources.

Procedure

1. **Centrifuge the PAXgene Blood RNA Tubes for 10 minutes at 3000–5000 x *g* using a swing-out rotor.**

   Note: Use only round-bottomed tube adapters. Tubes may break during centrifugation if centrifuge adaptors with conical bottoms are used.

   To save time, the QIAsymphony SP can be set up (steps 7 to 10) during this centrifugation step.

2. **After centrifugation, remove the supernatant by decanting. Discard the supernatant, and save the pellet for resuspension in step 3.**
3. **Mix 280 µl buffer QSX2 with 20 µl proteinase K per sample.**

   Note: For 24 (48, 72) samples, mix 7 (14, 21) ml of buffer QSX2 with 500 (1000, 1500) µl proteinase K.

4. **Add 300 µl Buffer QSX2–proteinase K mixture per tube. Close the tubes with the Secondary Hemogard Closures provided, and thoroughly resuspend the pellet by vortexing. For resuspension use a multitube vortexer at full speed for 30 seconds or until the pellets are completely resuspended.**
5. **Remove and discard the closures.**
6. **Add 200 µl Buffer BR2 per tube and place them into the appropriate sample carrier.**
7. **Ensure that the QIAsymphony SP is switched on.**

   The power switch is located at the bottom left corner of the QIAsymphony SP.

8. **Ensure that the "Waste" drawer is prepared properly, and perform an inventory scan of the "Waste" drawer, including the tip chute and liquid waste. Replace the tip disposal bag if necessary.**

9. **Load the required reagent cartridge and consumables (see Table 1, page 13) into the "Reagents and Consumables" drawer, and perform an inventory scan of the "Reagents and Consumables" drawer.**

   You must scan the bar code of the Buffer RDD bottle before placing it into the "Reagents and Consumables" drawer. To do this, choose the "Bottle ID" button in the "Consumables" dialog box on the touchscreen. Buffer RDD is equivalent to Buffer QSX1, the name used in the QIAsymphony SP software.

10. **Load the required elution rack into the "Eluate" drawer.**

    Place the adapter required for the specific elution format onto the selected elution position. If Elution Microtubes CL are used in the eluate cooling position ("Elution Slot 1"), remove the lower plate from the elution microtube rack using a spatula. Do not load a 96-well plate onto "Elution slot 4".

11. **Load the samples (from step 6) into the "Sample" drawer.**

12. **Using the touchscreen, enter the required information for each batch of samples to be processed.**

    Enter the following information:

    - Sample information (change default tube format; choose the "Select all" button on the sample view screen, and select "BD # 762165 PAXgene RNA 16x100" from the "Tube Insert 00" sheet)
    - Protocol ("Assay Control Set") to be run
    - Elution volume and output position

    After information about the batch has been entered, the status changes from "LOADED" to "QUEUED". As soon as one batch is queued the "Run" button appears.

13. **Press the "Run" button to start processing.**

    All processing steps are fully automated. The time elapsed is displayed.

    At the end of the protocol run, the status of the batch changes from "RUNNING" to "COMPLETED".

    It is recommended to use the "Elution slot 1" because this slot is able to cool the eluates after the run is complete.

14. **After the QIAsymphony protocol finishes, remove the elution microtubes or 2 ml Sarstedt tubes containing the purified RNA, and seal them with the appropriate caps.**

    If the "Eluate" drawer is opened and not reclosed when a batch is running (e.g., if elution racks that contain eluates are removed), the run will be paused and an inventory scan of the "Eluate" drawer will be performed. A message window appears during the scan and must be closed (by pressing **Close**) before the run can be restarted.

    Result files are generated for each elution plate.

15. **Follow step 15a if the RNA was eluted into Elution Microtubes CL or step 15b if the RNA was eluted into 2 ml Sarstedt tubes.**

    If using Elution Microtubes CL, heat an incubator to 80°C for use in step 15a. If using 2 ml Sarstedt tubes, heat a shaker–incubator, heating block, or water bath to 65°C for use in step 15b.

15a. **Place the elution microtube rack onto the PAXgene 96 Incubator Block preheated in the 80°C incubator, and incubate for 10 minutes at 80°C. Place a heavy plate over the caps to prevent them from popping open. After incubation, proceed immediately with step 16.**

    Note: Denaturation of the eluate is essential for maximum efficiency in downstream applications, such as RT-PCR, other amplification reactions, or cDNA synthesis. It is not necessary to denature samples more than once; samples remain denatured after freezing and thawing. Do not exceed the incubation time or temperature.

15b. **Incubate the eluates for 5 minutes at 65°C in a shaker–incubator, heating block, or water bath without shaking. After incubation, proceed immediately with step 16.**

    Note: Denaturation of the eluate is essential for maximum efficiency in downstream applications, such as RT-PCR, other amplification reactions, or cDNA synthesis. It is not necessary to denature samples more than once; samples remain denatured after freezing and thawing. Do not exceed the incubation time or temperature.

    If elution positions 2 or 3 were used (position 4 is not recommended for 96-well elution formats), you must remove the bottom plate of the elution microtube rack with a spatula first.

16. **After incubation, chill the elution microtubes or Sarstedt tubes immediately on ice. Put the bottom plate back onto the rack for storage. Store the purified RNA short-term at –20°C or at –80°C for longer time periods.**

    A temperature of –80°C is commonly recommended for storing isolated RNA. We also recommend storing RNA purified with the QIAsymphony PAXgene Blood RNA Kit at –80°C.

    For accurate quantification of RNA by absorbance at 260 nm, we recommend diluting the sample in 10 mM Tris-Cl,* pH 7.5 buffer. Dilution of the sample in RNase-free water may lead to inaccurately low values. Use the buffer in which the RNA is diluted to zero the spectrophotometer, and make sure to add the same volume of Buffer BR5 as the volume of eluted RNA to be diluted.

    Note: For quantification in Tris buffer, use the relationship A_{260} = 1 ⇒44 µg/ml.

    In general, magnetic particles are not carried over into eluates. If carryover does occur, magnetic particles in eluates will not affect most downstream applications. If magnetic particles need to be removed before performing downstream applications, tubes or plates containing eluates should be spun down, and eluates should be transferred to a clean tube. Alternatively, a suitable magnet can be used.

17. **If the reagent cartridge is only partially used, seal it with the Reuse Seal Strips (provided), and close the enzyme tubes with screw caps immediately after the end of the protocol run to avoid evaporation. Remove the enzyme rack and store it at 2–8°C.**

18. **Discard used sample tubes and waste according to your local safety regulations.**

    See page 9 for safety information.

19. **Clean the QIAsymphony SP.**

    Follow the maintenance instructions in the *QIAsymphony SP/AS User Manual — Operating the QIAsymphony SP*.

20. **Close the workstation drawers and switch off the QIAsymphony SP.**

* 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 problems that may arise. For more information, see also the Frequently Asked Questions on the respective product page at www.preanalytix.com or our Technical Support Center at **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 page 31 or visit **www.preanalytix.com**).

	Comments and suggestions
General handling	
a) Error message displayed in the touchscreen	If an error message is displayed during a protocol run, consult the *QIAsymphony SP/AS User Manual — Operating the QIAsymphony SP*.
RNA degraded	
a) RNase contamination	Although all buffers have been tested and guaranteed RNase-free, RNases can be introduced during use. Be certain not to introduce any RNases during the procedure or later handling. See Appendix A (page 23). Do not put RNA samples into a vacuum dryer that has been used in DNA preparation where RNases may have been used.
b) Incorrect blood donation	Strictly follow the instructions in the PAXgene Blood RNA Tubes Product Circular.
RNA does not perform well in downstream applications	
a) Salt carryover during elution	Ensure that the reagent cartridge is at room temperature (15–25°C).
b) Eluate concentrated by vacuum centrifugation	Do not concentrate the eluate by vacuum centrifugation (e.g., in a SpeedVac® or similar instrument). This can introduce RNases, lead to degradation due to high temperatures, and concentrate salts in the eluate, which can interfere with downstream applications.

	Comments and suggestions
c) Insufficient RNA used in downstream application	Quantify the purified RNA by spectrophotometric measurement of the absorbance at 260 nm (see Appendix B, page 25).
d) Excess RNA used in downstream application	Excess RNA can inhibit some enzymatic reactions. Quantify the purified RNA by spectrophotometric measurement of the absorbance at 260 nm (see Appendix B, page 25).
e) Bead carryover	In general, magnetic particles are not carried over into eluates. If carryover does occur, magnetic particles in eluates will not affect most downstream applications. If very high portions of eluates are needed for specific downstream assays, eluates could be spun down, and eluates should be transferred to a clean tube.
Low RNA yield	
a) Less than 2.5 ml blood collected in the PAXgene Blood RNA Tube	Ensure that 2.5 ml blood is collected in the PAXgene Blood RNA Tube (see the *PAXgene Blood RNA Tube Product Circular*).
b) RNA concentration measured in water	RNA concentration should be measured in 10 mM Tris-Cl, pH 7.5, for accurate quantification (see "Spectrophotometric quantification of RNA", page 25).
c) Pellet overdried in step 2	After removing the supernatant by decanting, it is sufficient to dab the rim of the tube 5–10 times with a clean paper towel. Excess drying (such as placing the tubes upside down in a rack or wiping the inner walls of the tube) is not recommended.
d) Blood incubated for <2 hours after collection	Incubate blood in the PAXgene Blood RNA Tube for at least 2 hours after collection. Incubation of the PAXgene Blood RNA Tube overnight may increase yields slightly in some cases.
e) Low white blood cell count	RNA yields are highly donor-dependent. Blood samples with low leukocyte counts (e.g., $<4.8 \times 10^6$ leukocytes/ml) will give low yields.

	Comments and suggestions
f) Magnetic particles were not completely resuspended	Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex for at least 3 minutes before use.
Low A_{260}/A_{280} value	
a) Wrong buffer used for RNA dilution	Use 10 mM Tris-HCl, pH 7.5, not RNase-free water, to dilute RNA before measuring purity (see "Purity of RNA" page 26).
b) Spectrophotometer not properly zeroed	To zero the spectrophotometer, use a blank containing the same proportion of elution buffer (Buffer BR5, provided in an extra bottle with the kit) and dilution buffers 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.
c) Absorbance reading at 320 nm was not subtracted from the absorbance readings at 260 nm and 280 nm	To correct for the presence of magnetic particles in the eluate, an absorbance reading at 320 nm should be taken and subtracted from the absorbance readings obtained at 260 nm and 280 nm (see "Quantification of RNA" page 25).
Precipitate in reagent trough of opened cartridge	
Buffer evaporation	Excessive evaporation can lead to increased salt concentration in buffers. Discard reagent cartridge. Make sure to seal buffer troughs of a partially used reagent cartridge with Reuse Seal Strips when not being used for RNA purification.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIAsymphony PAXgene Blood RNA Kits 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 QIAsymphony PAXgene Blood RNA 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 page 31 or visit **www.preanalytix.com**).

References

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

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

Contact Information

For technical assistance and more information, call one of the QIAGEN Technical Service Departments or local distributors (see page 31 or visit **www.qiagen.com**).

Appendix A: General Remarks on Handling RNA

Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. 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 and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep purified RNA on ice when aliquots are pipetted for downstream applications.

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 24). Alternatively, chloroform- resistant plasticware can be rinsed with chloroform* to inactivate RNases.

* 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.

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 hours) 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 with primary amines and cannot be used directly to treat Tris* buffers.

Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and CO_2. 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.

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

* 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.

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

Storage of RNA

Purified RNA may be stored in Buffer BR5 at –20°C for several weeks or –80°C for 1 or more years.

A temperature of –80°C is commonly recommended for storing isolated RNA. We also recommend storing RNA purified with the QIAsymphony PAXgene Blood RNA Kit at –80°C. Under these conditions, no degradation of RNA is detectable for over 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-based 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 28 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 total RNA", below). For small amounts of RNA, however, it may not be possible to accurately determine amounts photometrically. Small amounts of RNA can be quantified using the 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 ($A_{260} = 1 \Rightarrow 44$ 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 26), 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.

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, such as protein. However, the A_{260}/A_{280} value is influenced considerably by pH. Lower pH results in a lower A_{260}/A_{280} value and reduced sensitivity to protein contamination.† For accurate values, we recommend measuring absorbance in 10 mM Tris-Cl, pH 7.5. Pure RNA has an A_{260}/A_{280} value of 1.9–2.1‡ in 10 mM Tris·Cl, pH 7.5. Use the buffer in which the RNA is diluted to

* 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.

† 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.

zero the spectrophotometer, and make sure to add the same volume of Buffer BR5 as the volume of eluted RNA to be diluted. Buffer BR5 has high absorbance at 220 nm, which can lead to high background absorbance levels if the spectrophotometer is not properly zeroed.

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 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. QuantiTect® Primer Assays from QIAGEN (**www.qiagen.com/GeneGlobe**) are designed for SYBR Green–based real-time RT-PCR analysis of RNA sequences (without detection of genomic DNA) where possible. For real-time RT-PCR assays where amplification of genomic DNA cannot be avoided, the QuantiTect Reverse Transcription Kit provides fast cDNA synthesis with integrated removal of genomic DNA contamination (see Ordering Information, page 28).

Integrity of RNA

The integrity and size distribution of total RNA purified with QIAsymphony PAXgene Blood RNA Kit can be checked by denaturing agarose gel electrophoresis and ethidium bromide† staining or by using the 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.

Ordering information

Product	Contents	Cat. no.
QIAsymphony PAXgene Blood RNA Kit (96)	For 96 preps: 2 Reagent Cartridges, Enzyme Racks, Accessories, and RNase-Free Buffers	762635
PAXgene Blood RNA Tubes (100)	100 blood collection tubes; sold by BD	762165
QIAsymphony SP	QIAsymphony sample prep module, 1 year warranty on parts and labor	9001297
Accessories		
PAXgene 96 Incubator Block	Block for denaturation of eluates in PAXgene procedures	9238279
Adapter, tubes, 2 ml, Qsym	Non-cooling adapter for 2 ml screw-cap tubes; for use with the QIAsymphony SP tube carrier	9021670
Cooling Adapter, 2 ml, v2, Qsym	Cooling adapter for 2 ml screw-cap tubes; for use with the QIAsymphony SP/AS instruments (software version 3.1 and higher)	9020674
Sample Prep Cartridges, 8-well (336)	8-well Sample Prep Cartridges for use with the QIAsymphony SP	997002
8-Rod Covers (144)	8-Rod Covers for use with the QIAsymphony SP	997004
Filter-Tips, 1500 µl (1024)	Sterile, Disposable Filter-Tips, racked; (8 x 128)	997024
Filter-Tips, 200 µl (1024)	Sterile, Disposable Filter-Tips, racked; (8 x 128)	990332
Elution Microtubes CL (24 x 96)	Nonsterile polypropylene tubes; 2304 in racks of 96; includes cap strips	19588
QIAsymphony AS	QIAsymphony assay setup module, 1 year warranty on parts and labor	9001301
QIAsymphony Cabinet SP	Accessory for correct positioning of the QIAsymphony SP instruments	9020244

Product	Contents	Cat. no.
12-Tube Magnet	Magnet for separating magnetic beads in 12 x 1.5 ml or 2 ml tubes	36912
Related products		
QIAsymphony RNA Kit — for purification of total RNA from animal and human cells and tissues using the QIAsymphony SP		
QIAsymphony RNA Kit	For 192 preps: 2 Reagent Cartridges, Enzyme Racks, and Accessories	931636
QuantiTect Kits		
QuantiTect Reverse Transcription Kit (50)	For 50 x 20 µl reverse-transcription reactions: gDNA Wipeout Buffer, Quantiscript® Reverse Transcriptase, Quantiscript RT Buffer, RT Primer Mix, RNase-Free Water	205311
QuantiTect Reverse Transcription Kit (200)	For 200 x 20 µl reverse-transcription reactions: gDNA Wipeout Buffer, Quantiscript Reverse Transcriptase, Quantiscript RT Buffer, RT Primer Mix, RNase-Free Water	205313
QuantiTect Primer Assay (200)*	For 200 x 50 µl reactions or 400 x 25 µl reactions: 10x QuantiTect Primer Assay (lyophilized)	Varies
miScript PCR System — for quantification of mature miRNA, precursor miRNA, and mRNA from the same cDNA sample		
miScript Reverse Transcription Kit (50)	For 50 reactions: miScript Reverse Transcriptase Mix, miScript RT Buffer, RNase-Free Water	218061
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.

Product	Contents	Cat. no.
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
miScript SYBR Green PCR Kit (1000)	For 1000 reactions: QuantiTect SYBR Green PCR Master Mix, miScript Universal Primer	218075
Human miScript Assay 96 Set V13.0 (20)	846 miScript Primer Assays (for 20 x 50 µl reactions) targeting human miRNAs in miRBase version 13.0; provided in 96-well plates; 6 miScript PCR Controls on each plate	218421
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
Rotor-Gene Q — for outstanding performance in real-time PCR		
Rotor-Gene Q 5plex HRM System	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9001650

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

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