May 2015

QIAsymphony® SP Protocol Sheet

PAX_RNA_V4 and
PAX_RNA_V5

Sample to Insight

QIAGEN

General information

These protocols are for purification of total RNA, including miRNA (and other small RNAs), from human whole blood stabilized in PAXgene® Blood RNA Tubes using the QIAsymphony SP and the QIAsymphony PAXgene Blood RNA Kit.

Note: The PAX_RNA_V4 and V5 protocols must be used with QIAsymphony PAXgene Blood RNA Kits with cat. no. 762635. Older kits, with cat. no. 762535, cannot not be used with either protocol PAX_RNA_V4 or protocol PAX_RNA_V5.

Note: It is the user's responsibility to validate performance using this combination for any procedures used in their laboratory. For Research Use Only. Not for use in diagnostics procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Kit	QIAsmphony PAXgene Blood RNA Kit (cat. no. 762625)
Sample material	Human whole blood in PAXgene Blood RNA Tubes
Protocol names	PAX_RNA_V4 and PAX_RNA_V5
Default Assay Control Set	ACS_PAX_RNA_V4 and ACS_PAX-RNA_V5
Editable	Elution volume: 80 µl, 120 µl, 200 µl
Required software version	Version 4.0 or higher

Materials required but not provided

- PAXgene Blood RNA Tubes (BD™ cat. no. 762165)
- PAXgene 96 Incubator Block (cat. no. 9238279)

"Sample" drawer

Sample type	Human whole blood in PAXgene Blood RNA Tubes
Sample amount	2.5 ml human whole blood with a white blood cell count (WBC) between 4.8 x 10^6 and 1.1 x 10^7 cells per ml
Sample volume	Pellet resuspended in 500 µl Buffer QSX2-proteinase K-Buffer BR2 mixture (see *QIAsymphony PAXgene Blood RNA Kit Handbook* p.17, step 1–6)
Primary sample tubes	PAXgene Blood RNA Tubes
Secondary sample tubes	Not recommended
Inserts	For more information, see the "Product Resources" tab at **www.qiagen.com/Products/Catalog/Automated-Solutions/Sample-Prep/QIAsymphony-PAXgene-Blood-RNA-Kit**

"Reagents and Consumables" drawer

Position A1 and/or A2	Reagent cartridge
Position B1	QSX1 (RDD) Buffer bottle (bar code must be scanned)
Tip rack holder 1–17	Disposable filter-tips, 200 µl or 1500 µl
Unit box holder 1–4	Unit boxes containing sample prep cartridges or 8-Rod Covers

"Waste" drawer

Unit box holder 1–4	Empty unit boxes
Waste bag holder	Waste bag
Liquid waste bottle holder	Empty liquid waste bottle

"Eluate" drawer

Elution rack (we recommend using slot 1, cooling position)	We recommend using Elution Microtube CL (provided) or 2 ml Sarstedt® tubes. For more information, see the "Product Resources" tab at **www.qiagen.com/QIAsymphonyRNAKit**

Required plasticware

	24	48	72
Reagent cartridges	1	1	2
Sample prep cartridges*	27	54	81
8-Rod Covers†	3	6	9
Disposable filter-tips, 1500 µl‡	132(5)	264(9)	396(13)
Disposable filter-tips, 200 µl‡	28(1)	56(2)	84(3)

* 28 sample prep cartridges/unit box.

† Twelve 8-Rod Covers/unit box.

‡ 32 filter-tips/tip rack; the inventory scan requires additional tips (two 200 µl and nine 1500 µl tips). In parenthesis is the number of filled tip racks that cover the needed number of filter-tips.

Note: Numbers of filter-tips given may differ from the numbers displayed in the touchscreen depending on settings. We recommend loading the maximum possible number of tips.

Elution volume

The elution volume is selected on the touchscreen. Depending on the white blood cell count (WBC) of the sample, the final eluate volume may vary by up to ±10 µl compared to the selected volume. Elution in smaller volumes increases the final RNA concentration, but reduces yield* and increases variability of eluate volume. We recommend using the smallest elution volume only when the intended downstream application requires a higher RNA concentration.

Preparation of kit components and sample material, and instrument loading

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.

Important points before starting

- Blood must be collected in PAXgene Blood RNA Tubes.
- Use of a step dispenser, such as the Multipette plus from Eppendorf, and a multitube vortexer, such as the VX2500 from VWR, is recommended.

* For yield distribution, see the Technical Note *Typical total RNA Yields from PAXgene Blood RNA Tubes processed with the QIAsymphony PAXgene Blood RNA Kit* under the "Resources" tab at **www.preanalytix.com/products/blood/RNA/qiasymphony-paxgene-blood-rna-kit**

- All steps of the QIAsymphony PAXgene Blood RNA protocol for purification of total RNA, including miRNA, should be performed at 18–25°C.

Things to do before starting

- After blood collection, incubate PAXgene Blood RNA Tubes for at least 2 h at room temperature (15–25°C) before RNA purification. Incubation of PAXgene Blood RNA Tubes 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 h 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) by adding 900 µl RNase-free water (provided) to each. 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 (labeled ETDN) at position 3 of the enzyme rack on the reagent cartridge. DNase I solution can be stored at 2–8°C for up to 6 weeks.
- Please note that Buffer RDD provided in the QIAsymphony PAXgene Blood RNA Kit, is equivalent to Buffer QSX1, the name used in the QIAsymphony SP software. If you are using the Wizard or if Buffer RDD has not been scanned and correctly placed, the QIAsymphony SP will request that you place Buffer QSX1 on the instrument. In this case, place Buffer RDD from the QIAsymphony PAXgene Blood RNA Kit in the position specified by the QIAsymphony SP for Buffer QSX1.
- Buffer BR2 (reagent trough 1 and separate bottle) may form a precipitate upon storage. If necessary, 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 particles. Make sure that the piercing lid is placed on the reagent cartridge. If using a previously used reagent cartridge, make sure that the Reuse Seal Strips and trough cover have been removed.
- The enzyme rack must be attached to the reagent cartridge, and the tube caps must be removed.

* Kunitz units are the commonly used units for measuring DNase I, defined as the amount of DNase I that causes an increase in A260 of 0.001 per minute per milliliter at 25°C, pH 5.0, with highly polymerized DNA as the substrate (Kunitz, M. (1950) J. Gen. Physiol.**33**, 349 and 363).

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

Procedure

1. Centrifuge the PAXgene Blood RNA Tubes for 10 min 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. Per sample, mix 280 µl Buffer QSX2 with 20 µl proteinase K.

   Note: For 24 (48, 72) samples, mix 7 (14, 21) ml 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 s 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. Make sure that the QIAsymphony SP is switched on.

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

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

9. Load the required reagent cartridge and consumables 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.

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 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 the **Close** button) 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 min at 80°C. Place a heavy plate over the caps to prevent them from popping open. After incubation, proceed immediately with step 16.

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

    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 min 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.

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 at –15°C to –30°C or 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 (provided in a separate bottle) as the volume of eluted RNA to be diluted.

    Note: For quantification in Tris buffer, use the relationship A260 = 1 ⇒ 44 µg/ml.

* 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.

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 (see Appendix B of the *QIAsymphony PAXgene Blood RNA Kit Handbook*).

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 pages 8–10 of the *QIAsymphony PAXgene Blood RNA Kit Handbook* 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.

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