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QIASymphony[®] PAXgene[®] Blood ccfDNA Kit Instructions for Use (Handbook)



192

Version 1



For In Vitro Diagnostic Use



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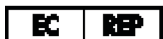


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Intended Use

The QIASymphony PAXgene Blood ccfDNA Kit, to be used with the QIASymphony SP instrument, is intended for automated isolation and purification of circulating cell-free DNA (ccfDNA) from plasma generated from human venous whole blood collected in the PAXgene Blood ccfDNA Tube.

The QIASymphony PAXgene Blood ccfDNA Kit utilizes magnetic-particle technology for automated isolation and purification of ccfDNA from human plasma.

The QIASymphony PAXgene Blood ccfDNA Kit is for *in vitro* diagnostic use and to be used by professional users, such as technicians and physicians who are trained in molecular biology techniques.

Intended User

This kit is intended for professional use.

The product is to be used only by personnel specifically instructed and trained in molecular biology techniques and familiar with this technology.

Description and Principle

Summary and explanation

Circulating cell-free DNA (ccfDNA) is present in plasma usually as short fragments (<1000 bp). The concentration of ccfDNA in plasma is usually low (can range from 1 to 100 ng/ml) and varies considerably between individuals. The CE-marked PreAnalytiX PAXgene Blood ccfDNA Tube in combination with the QIASymphony PAXgene Blood ccfDNA Kit constitutes a standardized workflow for collection, storage, and transport of blood; the stabilization of DNA in a closed tube; and the subsequent ccfDNA isolation and purification from human plasma using the QIAGEN® QIASymphony SP instrument.

Protocols on the QIASymphony SP instrument are provided for extraction of ccfDNA from 2.4 and 4.8 ml plasma generated from PAXgene Blood ccfDNA Tubes by double centrifugation. Plasma is transferred and processed in a secondary tube on the QIASymphony SP instrument.

Alternatively, primary tube handling protocols on the QIASymphony SP instrument are available for 2.4 and 4 ml plasma input volume. In this case there is no need for a second centrifugation step or plasma transfer into a secondary tube.

The QIASymphony PAXgene Blood ccfDNA Kit magnetic-particle technology enables purification of high-quality ccfDNA that is free of proteins, nucleases, and other impurities. The QIASymphony SP instrument performs all steps of the purification procedure. Up to 96 samples, in batches of 24, are processed in a single run. For isolation of genomic DNA (gDNA) from the nucleated cellular fraction of blood collected into PAXgene Blood ccfDNA Tubes on the QIASymphony SP instrument, refer to the instructions provided in the PAXgene Blood ccfDNA Tube Instructions for Use (www.PreAnalytiX.com).

Principles of the Procedure

The QIASymphony SP instrument technology combines the speed and efficiency of anion exchange-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 and consists of 3 steps: bind, wash, and elute (Figure 2). Users can choose between different sample input volumes.

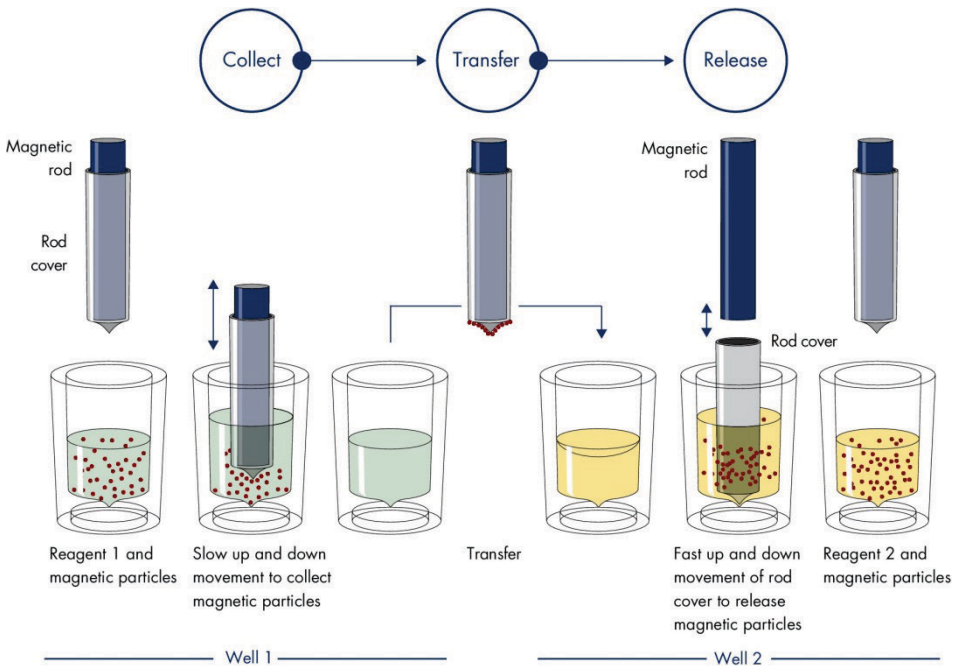


Figure 1. Schematic diagram of the QIASymphony SP instrument principle. The QIASymphony SP instrument processes a sample containing magnetic particles as follows: a magnetic rod protected by a rod cover enters a well containing the sample and attracts the magnetic particles. The magnetic rod cover is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

QIAsymphony PAXgene Blood ccfDNA Kit

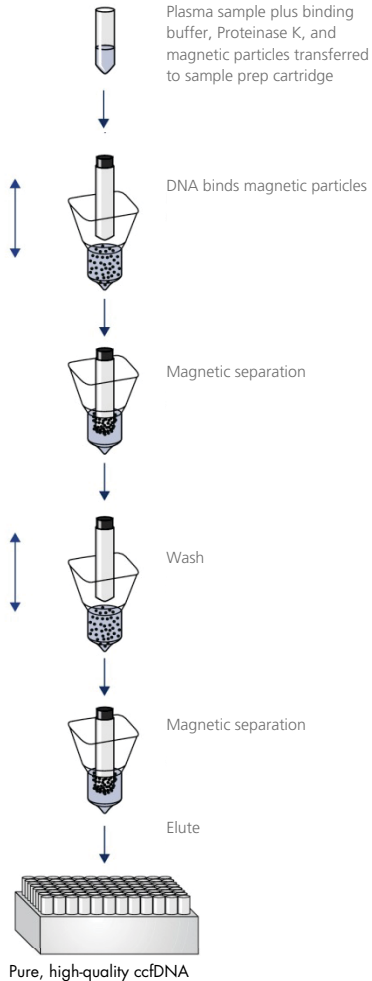


Figure 2. Steps for extraction of ccfDNA with the QIAsymphony PAXgene Blood ccfDNA Kit. ccfDNA fragments are isolated from plasma generated from human venous whole blood collected in the PAXgene Blood ccfDNA Tube. During the first processing step, plasma proteins are digested by Proteinase K while the ccfDNA binds to the surface of magnetic particles. Three wash steps guarantee that contaminants are removed. Finally, ccfDNA is eluted from the magnetic particles and is ready to use in downstream applications.

Materials Provided

Kit contents

QIASymphony PAXgene Blood ccfDNA Kit (CE-IVD) Catalog no. Number of reactions		(192) 768566 192		
Abbreviation	Identity	Quantity	Active ingredients	Concentration [%]**
RC	Reagent cartridge*†	2	Nonionic detergent Anion exchange magnetic particle NaOH Ethanol	≥0.5 – <10 [w/w] — ≥0.05 – <0.1 [w/w] ≥70 – <90 [v/v]
PROTK	Proteinase K†	5 x 10 ml	Proteinase K	≥1 – <3 [w/w]
PL	Piercing lid	2	—	—
RSS	Reuse Seal Set‡	2	—	—
	Elution Microtubes CL, racked#	2	—	—
	Caps for Elution Microtubes#	1 x (55 x 8)	—	—
	Instructions for Use (Handbook)	1	—	—
	PAXgene Blood ccfDNA Purification Protocol Selection Tool	1	—	—

* Contains sodium azide as a preservative.

† See page 36 for a list of symbols and definitions.

‡ A Reuse Seal Set contains 8 Reuse Seal Strips.

Also available separately, see Ordering Information.

** Maximum concentration in single well.

Materials Required but Not Provided

When working with chemicals and biological samples, always follow universal precautions and wear a suitable lab coat, disposable gloves, and protective goggles according to policies and procedures of your facility. For more information, please consult the appropriate safety data sheets (SDSs) available from the product supplier.

Make sure that instruments have been checked and calibrated according to the manufacturer's recommendations.

- Sample Prep Cartridges, 8-well (QIAGEN, cat. no. 997002)
- 8-Rod Covers (QIAGEN, cat. no. 997004)
- Filter-Tips, 200 µl and 1500 µl (QIAGEN, cat. nos. 990332 and 997024, respectively)
- Tip Disposal Bags (QIAGEN, cat. no. 9013395)
- PAXgene Blood ccfDNA Tubes (CE-IVD) (PreAnalytiX, cat. no. 768165)
- Sample tubes. For compatible primary and secondary tube formats, see the Labware list, which can be found under the Product Resource tabs and Resources tabs of the product pages on www.qiagen.com and www.PreAnalytiX.com, respectively.
- For compatible elution tube formats, see the Labware list which can be found under the Product Resource tabs and Resources tabs of the product pages on www.qiagen.com and www.PreAnalytiX.com, respectively.

Equipment*

- Pipet (5 ml)
- QIASymphony SP instrument (QIAGEN, cat. no. 9001297)

* Prior to use, ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

Warnings and Precautions

For in vitro diagnostic use

Read all instructions carefully before using the kit.

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

Safety information

When working with chemicals and biological samples, always follow universal precautions and wear a suitable lab coat, disposable gloves, and protective goggles according to policies and procedures of your facility. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each PreAnalytiX kit and kit component.

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

Precautions

Buffers in the reagent cartridge (RC) contain sodium azide. If buffers of the kit are spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water and then with 1% (v/v) sodium hypochlorite (bleach).

The following hazard and precautionary statements apply to components of the QIASymphony PAXgene Blood ccfDNA Kit.

MBS3

Contains: Sodium azide. Warning! May be harmful if swallowed. Wear protective gloves/protective clothing/eye protection/face protection.

Proteinase K



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

QSE2



Contains: Sodium hydroxide. Danger! Causes severe skin burns and eye damage. Dispose of contents/container to an approved waste disposal plant. **If in eyes:** Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. **If on skin (or hair):** Remove/take off immediately all contaminated clothing. Rinse skin with water/shower. Immediately call a poison center or doctor/physician. Store locked up. Wear protective gloves/protective clothing/eye protection/face protection.

QSW9



Contains: Ethanol. Danger! Highly flammable liquid and vapor. Causes serious eye irritation. Keep away from heat/sparks/open flames/hot surfaces. No smoking. Wear protective gloves/protective clothing/eye protection/face protection.

Reagent Storage and Handling

The QIASymphony PAXgene Blood ccfDNA Kit should be stored upright at room temperature (15–25°C). The magnetic particles in the reagent cartridges (RCs) remain active when stored within this temperature range.

Note: The label on the QIASymphony PAXgene Blood ccfDNA Kit box lists the expiration date of the kit. The expiration date is for the reagent cartridge.

Do not use the QIASymphony PAXgene Blood ccfDNA Kit once it has expired.

Kit components

The QIASymphony PAXgene Blood ccfDNA Kit contains ready-to-use Proteinase K solution that can be stored at room temperature (15–25°C).

Do not store RCs at temperatures below 15°C.

Open QIASymphony PAXgene Blood ccfDNA Kit RCs can be stored at room temperature (15–25°C) for up to 4 weeks, enabling cost-efficient reuse of reagents and more flexible sample processing, if properly resealed after use. If an RC is partially used, replace the cover of the trough containing the magnetic particles and seal the RC with the provided Reuse Seal Strips immediately after the end of the protocol run to avoid evaporation.

To avoid reagent evaporation, the RC should be open for a maximum of 15 hours (including run times) at a maximum environmental temperature of 32°C. Incorrect storage of the kit components can lead to accelerated aging of buffers.

Running batches with low sample numbers (<24) will increase both the time that the RC is open and the required buffer volumes, potentially reducing the total number of sample preparations possible per cartridge.

Avoid exposure of the RCs to UV light (e.g., used for decontamination) as exposure may cause accelerated aging of the RCs and buffers.

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

Specimen Collection and Preparation

The purification procedure is optimized for use with plasma generated from blood collected in PAXgene Blood ccfDNA Tubes. For information on blood collection, handling of these tubes and plasma preparation see the PAXgene Blood ccfDNA Tube Instructions for Use which can be found under the Product Resource tabs and Resources tabs of the product pages on www.qiagen.com and www.PreAnalytiX.com, respectively.

Plasma preparation can be done using (A) the standard double-centrifugation protocol or (B) via primary tube handling: direct processing of the one-time centrifuged PAXgene Blood ccfDNA Tubes on the QIASymphony SP instrument.

A) Plasma preparation from blood for standard protocols

1. Centrifuge the PAXgene Blood ccfDNA Tube at room temperature (15–25°C) for 15 min at 1600–3000 x *g* (first centrifugation) using a balanced swing out bucket centrifuge. If braking is preferred, it is recommended to use medium level braking, and it should be validated for your specific workflow.

Note: For best performance for samples stored refrigerated prior to centrifugation, remix the sample by inverting three times and allow the tubes to return to room temperature before processing.

2. Pipet the plasma into a 15 ml conical bottom centrifuge tube (not provided), making sure to not disturb the nucleated cellular fraction.
3. Centrifuge the 15 ml conical bottom centrifuge tube at room temperature (15–25°C) for 10 min at 1600–3000 x *g* (second centrifugation) using a balanced centrifuge.

Note: Do not exceed the secondary tube manufacturer's maximum recommended centrifugation speed.

4. Pipet the required plasma volume (see section “Sample volume”, page 21) into a 14 ml, 17 x 100 mm polystyrene, round-bottom tube, making sure to not disturb the residual blood cell pellet, if present.
5. Transfer the round-bottom tube with the plasma sample into the tube carrier and load the tube carrier in the sample input drawer of the QIAasymphony SP instrument.

Note: For maximum ccfDNA yield, process the maximum volume of plasma available.

Note: Prevent formation of foam in or on the surface of plasma samples during pipetting. Foam or air bubbles on samples can lead to pipetting of wrong sample volume.

Note: After transfer of plasma in secondary tube ccfDNA is stable in plasma at 15–25°C for up to 3 days or at 2–8°C for up to 7 days. For longer storage, we recommend freezing aliquots at –20°C or –80°C.

Note: When using previously stored plasma samples (e.g., stored at 2–8°C or frozen at –20°C or –80°C), these should be equilibrated to room temperature (15–25°C) before starting the run; for frozen samples, please see section “C)

Freezing and thawing of plasma samples processed from the PAXgene Blood ccfDNA Tube”.

B) Plasma preparation from blood for primary tube handling on the QIASymphony SP instrument

1. Centrifuge the PAXgene Blood ccfDNA Tube at room temperature (15–25°C) for 15 min at 3,000 x *g* using a balanced swing out bucket centrifuge. If braking is preferred, it is recommended to use medium level braking and it should be validated for your specific workflow.

Note: For best performance for samples stored refrigerated prior to centrifugation, remix the sample by inverting three times and allow the tubes to return to room temperature before processing.

Quantify the plasma volume in each tube after removal from the centrifuge bucket with the PAXgene Blood ccfDNA Purification Protocol Selection Tool provided as a kit content (Figure 3). Upon removal of the tube from the centrifuge, the teal arrow on the tool is aligned with the plasma/cell interface. The blue lines indicate if the plasma level is sufficient for the 2.4 or 4.0 ml primary tube handling protocol. A minimum plasma column height of 2.3 cm is needed for the 2.4 ml protocol and a minimum of 3.4 cm is needed for the 4.0 ml protocol.

Determine the optimal protocol to process
PAXgene Blood ccfDNA Tubes (CE-IVD)
directly on the QIASymphony SP instrument

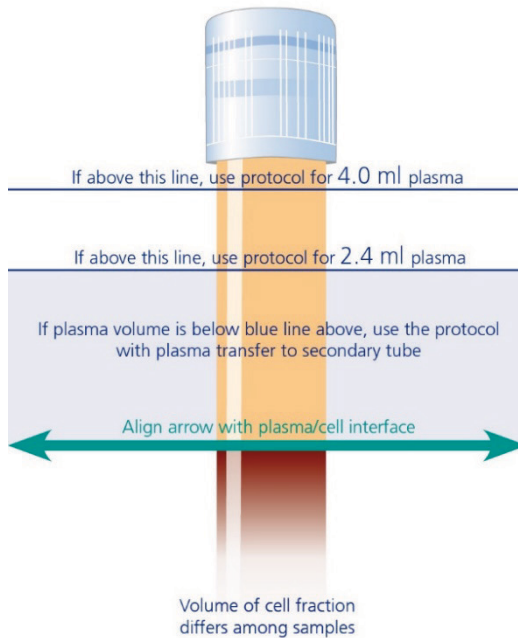


Figure 3. Plasma volume determination using the PAXgene Blood ccfDNA Purification Protocol Selection Tool (This is for illustration purpose only; please do not print because the actual size differs – not for use with samples).

Note: In case a clear separation of plasma and cell fraction did not occur or phases were accidentally mixed upon removal from the centrifuge, centrifugation should be repeated.

Note: Check for a clear separation before placing the tube on the instrument.

2. Remove the Hemogard Closure Cap from the PAXgene Blood ccfDNA Tubes before placement on the QIASymphony SP instrument for direct ccfDNA extraction.

Note: Removing tube closure and handling of open tubes should be done carefully to reduce potential risk of sample spillage, cross contamination between tubes and blood exposure.

3. Place the opened PAXgene Blood ccfDNA Tubes that contain sufficient plasma into the tube carrier and load the tube carrier in the sample input drawer of the QIASymphony SP instrument.

C) Freezing and thawing of plasma samples processed from the PAXgene Blood ccfDNA Tube

1. For freezing, transfer the plasma into suitable tubes (e.g., cryovials) placed in an appropriate rack.
2. Freeze and store the plasma at -20°C . For storage below -20°C , freeze the plasma samples first at -20°C for at least 24 h, then transfer it to -80°C .
3. Thaw the plasma at room temperature ($15\text{--}25^{\circ}\text{C}$).

Note: Do not thaw at low temperatures (e.g., 4°C).

4. If cryoprecipitates form in the plasma, vortex the tube for 30 s after thawing and use the sample for the QIASymphony SP instrument ccfDNA isolation procedure without further treatment.

Note: It is not recommended to centrifuge the plasma to remove cryoprecipitates because they may contain ccfDNA.

Note: To avoid formation of cryoprecipitates, plasma can be thawed at 30°C for 30 min instead of room temperature.

Procedure

Overview: Automated purification of ccfDNA on the QIASymphony SP instrument

The QIASymphony SP instrument 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 before a run. Start the protocol and remove purified ccfDNA from the “Eluate” drawer after processing. Refer to the user manuals supplied with your instrument for operating instructions.

Note: Optional maintenance is not mandatory for instrument function, but is highly recommended to reduce risk of contamination.

Sample volume

To make sure that in the regular workflow 2.4 ml (PAXcircDNA_2400 protocol) and 4.8 ml sample (PAXcircDNA_4800 protocol) are transferred by the instrument, a void volume of 0.4 and 0.5 ml, respectively, is required, which means a minimum of 2.8 and 5.3 ml sample input must be provided. If the available plasma volume is lower than 2.8 or 5.3 ml, **Less Sample mode**, an integrated function of the protocol, automatically allows the transfer of lower plasma volumes than listed. In this case, less sample is transferred by the instrument. The difference of the plasma volume pipetted will be recorded in the result file. In addition, the respective samples are flagged as **unclear** (error code 140043, **Enable Less Sample mode**). The minimum plasma input volumes to enable **Less Sample mode** are 1.6 and 4.1 ml. Samples will not be processed and will be flagged **invalid** if less sample volume is provided. For the primary tube handling workflow, the appropriate sample volume is assured by using the PAXgene Blood ccfDNA Purification Protocol Selection Tool provided as a kit content (described under “Preparation of sample material” on page 25).

Loading RCs into the “Reagents and Consumables” drawer

Reagents for purification of ccfDNA are contained in an innovative reagent cartridge (RC) (Figure 4). Each trough of the RC contains a particular reagent, such as magnetic particles, binding buffer, wash buffer, or elution buffer. Partially used RCs can be reclosed with Reuse Seal Strips for later reuse, which avoids generation of waste due to leftover reagents at the end of the purification procedure.



Figure 4. QIASymphony reagent cartridge (RC). The RC contains all reagents required for the protocol run.

Before starting the procedure, make sure that the magnetic particles are fully resuspended. Remove the magnetic-particle trough from the RC frame, vortex it vigorously for at least 3 min then replace it in the RC frame before the first use.

Note: Magnetic particles can change color. This has no influence on performance.

Place the RC into the RC holder. Before using a RC for the first time, place the piercing lid on top of the RC (Figure 4).

Note: The piercing lid is sharp. Take care when placing it onto the RC. Make sure to place the piercing lid onto the RC in the correct orientation.

After the magnetic-particle trough cover is removed, the RC is subsequently loaded into the “Reagents and Consumables” drawer.

Partially used RCs can be stored until needed again (see “Reagent Storage and Handling” on page 14).

Note: Proteinase K must be added (see “Things to do before starting” on page 27).

Note: Make sure that RCs, magnetic-particle troughs and Proteinase K bottles are not interchanged between different kit lots.

Loading plastic ware into the “Reagents and Consumables” drawer

Sample prep cartridges, 8-Rod Covers (both preracked in unit boxes), and disposable Filter-Tips (200 µl tips provided in blue racks, 1500 µl tips provided in black racks) are loaded into the “Reagents and Consumables” drawer.

Note: Make sure that the covers of the unit boxes are removed before loading the unit boxes into the “Reagents and Consumables” drawer.

Note: Tips have filters to help prevent cross-contamination.

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

Note: Do not refill tip racks or unit boxes for Sample Prep Cartridges or 8-Rod Covers before starting another protocol run. The QIASymphony SP instrument can use partially used tip racks and unit boxes.

For plasticware ordering information, see page 39.

Loading the “Waste” drawer

Sample Prep Cartridges and 8-Rod Covers used during a run are reracked in empty unit boxes in the “Waste” drawer. Make sure that the “Waste” drawer contains sufficient empty unit boxes for plastic waste generated during the protocol run.

Note: Make sure 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.

Note: The presence of a tip disposal bag is not checked by the system. Make sure that the tip disposal bag is properly attached before starting a protocol run. For more information, see the user manuals provided with your instrument. Empty the tip bag after a maximum of 96 samples have been processed to avoid a tip jam.

A waste container collects liquid waste generated during the purification procedure. The “Waste” drawer can only be closed if the waste container is in place. Dispose of the liquid waste according to your local safety and environment regulations. Do not autoclave the filled waste bottle. Empty the waste bottle after a maximum of 96 samples have been processed.

Loading the “Eluate” drawer

Load the required elution rack into the “Eluate” drawer. As long-term storage of eluates in the “Eluate” drawer may lead to evaporation or condensation, the cooling position must be used. Only use “Elution slot 1” with the corresponding cooling adapter.

Selected elution volume (µl)*	Initial elution volume (µl)†
60	75

* This is the minimum accessible volume of eluate in the final elution tube for the QIAGEN EMT rack (cat. no. 19588) and 1.5 ml Sarstedt screw cap tubes (cat. no 72.607). In individual cases the final eluate volume for single samples may be up to 5 µl less.

† The initial volume of elution buffer required to ensure that the actual volume of eluate is the same as the selected volume.

Inventory scan

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

Preparation of sample material

See “Specimen Collection and Preparation”, page 16.

Storage of ccfDNA

After sample preparation, ccfDNA eluates can be stored at –20 or –80°C. Frozen eluates should not be thawed more than three times. For the most up to date information on ccfDNA stability in eluates refer to product page on www.qiagen.com or www.PreAnalytiX.com.

Protocol overview

Table 1. Protocol overview

Sample	Processing of PAXgene Blood ccfDNA Tube	Sample input volume (incl. void volume) (ml)	Sample volume used for ccfDNA extraction (ml)	Elution volume (µl)	QIASymphony SP instrument protocol
Plasma generated from human venous whole blood collected in PAXgene Blood ccfDNA Tubes	2x centrifugation, plasma transfer into secondary tube	2.8	2.4	60	PAXcircDNA_2400
		5.3	4.8	60	PAXcircDNA_4800
	1x centrifugation, direct processing on QIASymphony SP instrument	According to selection tool	2.4	60	PAXcircDNA PrimaryTube_2400
			4.0	60	PAXcircDNA PrimaryTube_4000

Important points before starting

- When working with chemicals and biological samples, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate SDSs.
- Make sure that you are familiar with operating the QIASymphony SP instrument. Refer to the user manuals supplied with your instrument for operating instructions.
- Before beginning the procedure, read the section “Principles of the Procedure”.
- Make sure that you are familiar with the Protocol sheet and the Labware list (can be found under the Product Resource tabs and Resources tabs of the product pages on www.qiagen.com and www.PreAnalytiX.com, respectively).
- Avoid vigorous shaking of the RC; otherwise, foam may be generated, which can lead to liquid-level detection problems.

- Blood must be collected in PAXgene Blood ccfDNA Tubes (CE-IVD; PreAnalytiX, cat. no. 768165). For blood collection and handling and plasma processing instructions, see the tube Instructions for Use that can be found under the **Product Resources** tab and Resources tab of the product page on www.qiagen.com or www.PreAnalytiX.com, respectively.

Things to do before starting

- Before starting the procedure, make sure that the magnetic particles are fully resuspended. Vortex the trough containing the magnetic particles vigorously for at least 3 min before first use.
- Make sure that the piercing lid is placed on the RC and the lid of the magnetic-particle trough has been removed or, if using a partially used RC, make sure the Reuse Seal Strips have been removed.
- Proteinase K is not included in the RC but has to be provided by the user (sample drawer, slot A, position 1 and/or 2). Make sure that correct Proteinase K volume is available.

The QIASymphony PAXgene Blood ccfDNA Kit contains ready-to-use Proteinase K solution. Proteinase K can be stored at room temperature (15–25°C). To store for extended periods of time, we suggest keeping the enzyme vials with Proteinase K at 2–8°C.

Sample number	PAXcircDNA_2400/PAXcircDNA PrimaryTube_2400* (µl)	PAXcircDNA_4800/PAXcircDNA PrimaryTube_4000* (µl)
8	1980	2860
24	3740	6380
48	6380	11660 [†]
96	11660 [†]	23320 [†]

* For each sample, 110 µl (for 2400 µl plasma) or 220 µl (for 4800/4000 µl plasma) are required, plus an additional void volume of 1100 µl [(n x 110 or 220 µl) + 1100 µl].

[†] If more than 11,660 µl are required, use a second tube (Corning, cat. no. 352051). For the second tube, an additional void volume of 1100 µl is required.

Note: Tubes containing Proteinase K are placed in a tube carrier. The tube carrier containing the Proteinase K must be placed on positions 1 and 2 in slot A of the “Sample” drawer. We recommend using 14 ml 17 x 100 mm polystyrene, round-bottom tubes (Corning, cat. no. 352051) for Proteinase K.

- If samples are barcoded, orient samples in the tube carrier so that the bar codes face the bar code reader at the left side of the QIASymphony SP instrument.
- For information about sample tubes and minimum sample volumes compatible with the protocols, see the corresponding labware list that can be found under the Product Resource tabs and Resources tabs of the product pages on www.qiagen.com and www.PreAnalytiX.com, respectively.

Protocol: Automated Purification of ccfDNA on the QIASymphony SP instrument

Detailed information for each protocol, including volumes and tubes, is provided in the Protocol sheet and Labware list which can be found under the Product Resource tabs and Resources tabs of the product pages on www.qiagen.com and www.PreAnalytiX.com, respectively. For preparation of the sample material (plasma generated from human venous whole blood collected in the PAXgene Blood ccfDNA Tube), see sections “Specimen Collection and Preparation” and “Preparation of sample material” in this handbook as well as the tube Instructions for Use which can be found under the **Product Resources** tab and Resources tab of the product page on www.qiagen.com or www.PreAnalytiX.com, respectively.

1. Close all drawers and the hood.
2. Power ON the QIASymphony SP instrument, and wait until the **Sample Preparation** screen appears and the initialization procedure has finished.
3. The power switch is located at the bottom, left corner of the QIASymphony SP instrument.
4. Log on to the instrument.

Load the required elution rack into the “Eluate” drawer.

Do not load a 96-well plate onto “Elution slot 4”. “Elution slot 1”, with the corresponding cooling adapter, must be used.

When using a 96-well plate, make sure that the plate is in the correct orientation, as incorrect placement may cause sample mix-up in downstream analysis.

When using the Elution Microtubes CL rack, remove the bottom by twisting the rack until the bottom comes off.

5. Make sure the “Waste” drawer is properly prepared and perform an inventory scan of the “Waste” drawer, including the tip chute, tip park station, empty liquid waste container, and empty unit boxes. Replace the tip disposal bag if necessary.

6. Load the required RC(s) and consumables into the “Reagents and Consumables” drawer.
7. Perform an inventory scan of the “Reagents and Consumables” drawer.
8. Place the samples into the appropriate sample carrier, and load them into the “Sample” drawer.

Note: In addition to regular processing including plasma transfer into an appropriate secondary tube (14 ml Falcon® polystyrene round-bottom tube 17 x 100 mm) placed into the appropriate sample carrier, the PAXgene Blood ccfDNA Tube primary tube handling workflow allows ccfDNA extraction directly from the PAXgene Blood ccfDNA Tube (10 ml PAXgene Blood ccfDNA Tube, 16 x 100 mm). For more information on primary tube handling, see the Protocol sheet, the Labware list, and tube Instructions for Use which can be found under the **Product Resources** tab and Resources tab of the product page on www.qiagen.com or www.PreAnalytiX.com, respectively.

9. Using the touchscreen, enter the required information for each batch of samples and for Proteinase K to be processed.

Enter the following information:

- Sample information (depending on sample racks used select BD #352051 FalconPP 17 x 100 or BD_#768165 PAXgene ccfDNA 16 x 100)
- Protocol to be run (Assay Control Set)
- 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.

10. Place the Proteinase K into the appropriate sample carrier on position 1 and 2, and load them into slot A of the “Sample” drawer.
11. Define the Proteinase K by pressing the **IC** button.
12. Press the **Run** button to start the purification procedure.

All processing steps are fully automated. At the end of the protocol run, the status of the batch changes from **RUNNING** to **COMPLETED**.

13. Retrieve the elution rack containing the purified ccfDNA from the “Eluate” drawer. Confirm the elution rack removal with the QIASymphony SP instrument software.

14. The ccfDNA is ready to use or can be stored at -20°C or -80°C for up to 6 months.

QIAGEN recommends removing the eluate plate from the “Eluate” drawer immediately after the run has finished. Depending on temperature and humidity, elution plates left in the QIASymphony SP instrument after the run is completed may experience condensation or evaporation.

Note: Using the cooling function of the QIASymphony SP instrument overnight runs are possible.

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 first be placed in a suitable magnet and the eluates transferred to a clean tube (see “Appendix: Quantification of ccfDNA”).

Result files are generated for each elution plate.

15. If an RC is only partially used, seal it with the provided Reuse Seal Strips after the end of the protocol run to avoid evaporation.

Note: For more information about storage of partially used RCs, see “Reagent Storage and Handling”.

16. Discard used sample tubes and waste according to your local safety regulations. See “Warnings and Precautions”, for safety information.

17. Clean the QIASymphony SP instrument.

Follow the maintenance instructions in the user manuals supplied with your instrument. Make sure to clean the tip guards regularly to minimize the risk of cross-contamination.

18. Close the instrument drawers and power OFF the QIASymphony SP instrument.

Quality Control

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

Limitations

System performance has been established in performance evaluation studies purifying ccfDNA from plasma generated from human venous whole blood collected in the PAXgene Blood ccfDNA Tube.

The performance characteristics for the QIASymphony PAXgene Blood ccfDNA Kit have been established with 18S ribosomal and DYS14 Y-chromosomal ccfDNA fragments in plasma.

It is the user's responsibility to verify system performance for any procedures used in their laboratory that are not covered by the QIAGEN and PreAnalytiX performance evaluation studies.

To minimize the risk of a negative impact on the diagnostic results, adequate controls for downstream applications should be used. Any diagnostic results that are generated must be interpreted in conjunction with other clinical or laboratory findings.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving problems that could arise. For contact information and a list of Frequently Asked Questions visit the respective product page on www.PreAnalytiX.com or www.qiagen.com.












		Comments and suggestions
General handling		
a)	Error message displayed on the touchscreen	If an error message is displayed during a protocol, refer to the user manuals supplied with your instrument.
b)	Varying eluate volume after overnight runs	To optimize throughput, the QIASymphony PAXgene Blood ccfDNA Kit supports processing samples overnight by cooling eluates on the QIASymphony SP instrument. During long cooling periods, eluate volumes can change depending on temperature and humidity in the laboratory. For example, if the chosen volume was 60 µl at 15–25°C and 30–60% humidity, the volume can be in the range of 50–85 µl after 12 hour storage on the instrument.
Cryoprecipitates after thawing of plasma samples		
a)	Cryoprecipitates form in the plasma	To avoid formation of cryoprecipitates, the plasma can be thawed at 30°C for 30 min instead of room temperature. Do not thaw at lower temperatures (e.g., 4°C). Vortex the tube for 30 seconds after thawing
b)	Low ccfDNA yield from plasma after removing cryoprecipitates	Do not centrifuge the plasma to remove cryoprecipitates, as they may contain ccfDNA.
Insufficient plasma volume from PAXgene Blood ccfDNA Tubes		
a)	Less than 10 ml blood collected in PAXgene Blood ccfDNA Tube	Ensure that 10 ml blood is collected in the PAXgene Blood ccfDNA Tube. See the PAXgene Blood ccfDNA Tube Instructions for Use.
b)	High cell fraction	High cell fraction (hematocrit) level in human whole blood (e.g., above 51% for male or 47% for female) can lead to decreased plasma volumes.
c)	Low plasma yield after centrifugation	Longer blood storage and transport times or different centrifugation conditions than specified can have an impact on plasma yields.

		Comments and suggestions
Precipitate in trough of opened reagent cartridge (RC) in the QIASymphony PAXgene Blood ccfDNA Kit		
a)	Buffer evaporation	Excessive evaporation may lead to increased salt concentration in buffers. Discard RC. Make sure to seal buffer troughs of a partially used RC with Reuse Seal Strips when not being used for purification.
b)	Storage of RC	Storage of RC below 15°C may lead to formation of precipitates.
Low yield of ccfDNA		
a)	Magnetic particles were not completely resuspended	Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex for at least 3 min before use. Incomplete resuspension may lead to pipetting errors.
b)	Clogging of pipet tip due to insoluble material	Insoluble material was not removed from the sample prior to starting the QIASymphony SP instrument purification procedure.
c)	Sample material contains low concentration of ccfDNA	Due to very low amounts of ccfDNA in plasma samples, measurement of ccfDNA concentration can be challenging depending on the quantification method used. Use of sensitive qPCR is recommended to check ccfDNA concentration in eluates.
d)	Incomplete resealing of RC	Exchange with surrounding air may lead to reduced stability of buffers leading to reduced efficiency of ccfDNA extraction with a partially used RC. Make sure to carefully seal buffer troughs of a partially used RC with Reuse Seal Strips when not being used for purification.
Low ccfDNA performance in downstream applications		
a)	Eluate concentrated by vacuum centrifugation	Do not concentrate the eluate by vacuum centrifugation (e.g., in a SpeedVac® or similar instrument). This can lead to degradation due to high temperatures and concentrated salts in the eluate, which can interfere with downstream applications.
b)	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. In case very high portions of eluates are needed for specific downstream assays, eluates can be spun down and transferred to a clean tube.

		Comments and suggestions
No/incomplete sample transfer		
a)	Incorrect sample volume is loaded for standard protocols	If less sample volume is loaded than specified, there is an increased risk of unclear flagging of the sample, or no transfer of the sample (invalid flagging). Load the correct sample volume as described in the corresponding Protocol sheet and Labware list.
b)	Bubbles and/or foam in sample tube	Bubbles or foam in the sample and/or sample input tube may result in incorrect liquid level detection and subsequent incomplete sample transfer. Remove bubbles from the sample tube.

Symbols

The following symbols may appear in the instructions for use or on the packaging and labeling. Additional symbols are explained in Kit contents (page 9).

Symbol	Symbol definition
 <N>	Contains reagents sufficient for <N> tests Indicates the total number of IVD tests that can be performed with the IVD.
	Use by
	In vitro diagnostic medical device
	CE Mark. This product fulfills the requirements of the European Regulation 2017/746 for in vitro diagnostic medical devices.
	Catalog number
	Lot number
	Material number (i.e., component labeling)
	Components (i.e., a list of what is included)
	Contains (contents)
	Number (i.e., vials, bottles)
	Global Trade Item Number
Rn	R is for revision of the Instructions for Use (Handbook) and n is the revision number



Temperature limitation



Manufacturer



Consult instructions for use



Caution



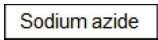
Proteinase K



Well number (i.e., RC well)



Reagent cartridge



Sodium azide



European authorized representative according to Regulation (EU) 2017/746

Appendix: Quantification of ccfDNA

Because of very low concentrations of ccfDNA in sample materials, measurement of DNA with a spectrophotometer is not recommended. For determination of concentration of ccfDNA, a sensitive and accurate fluorescence-based quantitation assay or a real-time PCR assay should be used.

If magnetic particles need to be removed, apply the tube containing the DNA to a suitable magnetic separator (e.g., QIAGEN 12-Tube Magnet, cat. no. 36912) until the magnetic particles are separated.

If DNA is in microplates, apply the microplate to a suitable magnetic separator (e.g., QIAGEN 96-Well Magnet Type A, cat. no. 36915) until the magnetic particles are separated. If a suitable magnetic separator is not available, centrifuge the tube containing the DNA for 1 minute at full speed in a microcentrifuge to pellet any remaining magnetic particles.

Ordering Information

Product	Contents	Cat. no.
PreAnalytiX		
QIASymphony PAXgene Blood ccfDNA Kit (CE-IVD) (192)	Includes 2 RCs, Proteinase K tubes and accessories	768566
PAXgene Blood ccfDNA Tubes (CE-IVD) (100)	100 Tubes: 16 x 100 mm, 1.5 ml Additive, 10 ml blood draw volume	768165
QIAGEN		
QIASymphony SP instrument	QIASymphony sample prep module, 1 year warranty on parts and labor	9001297
Related QIAGEN products		
Proteinase K (10 ml)	1 x 10 ml bottle	19133
Reagent Cartridge Holder (2)	RC holder for use with the QIASymphony SP instrument	997008
Cooling Adapter, 2 ml, v2, Qsym	Cooling adapter for 2 ml screw-cap tubes. For use in the QIASymphony SP instrument "Eluate" drawer	9020674
Cooling Adapter, EMT, v2, Qsym	Cooling adapter for EMT racks. For use in the QIASymphony SP instrument "Eluate" drawer	9020730
Cooling Adapter, Snap-Cap Microtube QIASymphony, Qsym	Cooling adapter for 1.5 ml Eppendorf® LoBind Snap Cap Safe-Lock tubes. For use in the QIASymphony SP instrument "Eluate" drawer	9020731
Sample Prep Cartridges, 8-well (336)	8-well sample prep cartridges for use with the QIASymphony SP instrument	997002
8-Rod Covers (144)	8-rod covers for use with the QIASymphony SP instrument	997004
Filter-Tips, 200 µl (1024)	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAcube® and the QIASymphony SP/AS instrument	990332
Filter-Tips, 1500 µl, Qsym SP (1024)	Disposable Filter-Tips, racked; (8 x 128). For use with the QIASymphony SP/AS instrument	997024
Tip Disposal Bags (15)	Tip disposal bags for use with the QIASymphony SP instrument	9013395

Product	Contents	Cat. no.
12-Tube Magnet	Magnet for separating magnetic particles in 12 x 1.5 ml or 2 ml tubes	36912
96-Well Magnet Type A	Magnet for separating magnetic particles in wells of 96-well plates, 2 x 96-Well Microplates FB	36915
Reuse Seal Set (20)	Reuse seal sets for sealing partly used QIASymphony RCs	997006
Elution Microtubes CL (24 x 96)	Non-sterile polypropylene tubes (0.85 ml maximum capacity, less than 0.7 ml storage capacity, 0.4 ml elution capacity); 2304 in racks of 96; includes cap strips	19588
Caps for Elution Microtubes (50 x 8)	Caps for Elution Microtubes (50 x 8)	19591
14 ml Falcon Tube	Polystyrene round-bottom tube 17 x 100 mm used as sample carrier for the QIASymphony SP instrument	Corning, 352051

Document Revision History

Revision	Description
R1, May 2021	Initial release
R2, May 2022	Removed "(CE-IVD)" in some occurrences. Updated the Kit Contents table. Included www.PreAnalytiX.com as to where related resources can be found. Specified "specimens" as "blood specimens" under Safety information section. Changed "Discard sample and assay waste" to "Discard biohazardous materials and kit waste" under Safer information section. Updated sections "A) Plasma preparation from blood for standard protocols" and "B) Plasma preparation from blood for primary tube handling on the QIASymphony SP instrument".

Notes

Notes



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

Explore more at: www.PreAnalytiX.com

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