QIAsymphony® PAXgene® Blood ccfDNA Kit Handbook

For purification of circulating cell-free DNA from plasma using the QIAsymphony SP instrument. To be used only in conjunction with PAXgene Blood ccfDNA Tubes.

When the QIAsymphony PAXgene Blood ccfDNA Kit is used in conjunction with plasma generated from PAXgene Blood ccfDNA Tubes, the system provides purified ccfDNA for research tests including but not limited to PCR and NGS.

The PAXgene Blood ccfDNA System is intended for Research Use Only. Not for use in diagnostic procedures.

August 2016

PreAnalytiX
A QIAGEN / BD Company

PreAnalytiX Company

PreAnalytiX GmbH

Feldbachstrasse

CH – 8634 Hombrechtikon

Switzerland

www.preanalytix.com

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

Kit Contents

QIAsymphony PAXgene Blood ccfDNA Kit	**(192)**
Catalog no.	**768536**
Number of preps	**192**
Proteinase K	5 × 10 ml
Reagent Cartridge*	2
Piercing Lid	2
Reuse Seal Set	2
Elution Microtubes CL, racked	2
Caps for Elution Microtubes†	2 × (55 × 8)
Handbook	1

* Prefilled reagent cartridges include buffers that contain ethanol. See page 10 for safety information.

† Also available separately. See page 27 for Ordering Information.

Reagent Storage and Handling

Store the QIAsymphony PAXgene Blood ccfDNA Kit at room temperature (15–25°C). Do not store reagents cartridges at temperatures below 15°C. When stored properly, the kit is stable until the expiration date on the kit box.

The QIAsymphony PAXgene Blood ccfDNA Kit contains a ready-to-use proteinase K solution in glass vials. 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. For the use on the QIAsymphony SP the proteinase K has to be filled into 14 ml Falcon tubes. Remaining proteinase K can be stored within these tubes with properly closed lid at 2–8°C.

Partially used reagent cartridges can be stored for a maximum of 12 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

Whole blood must be collected into PAXgene Blood ccfDNA Tubes (cat. no. 768115). See the *PAXgene Blood ccfDNA Tube Product Circular* for information about specimen collection and handling.

Blood samples filled into PAXgene Blood ccfDNA Tubes are stable at room temperature (15–25°C) for up to 7 days or at higher temperatures (up to 35°C) for up to 1 day until centrifugation and plasma processing. Plasma generation from blood collected into PAXgene Blood ccfDNA Tubes is described in "Preparation of sample material", page 19.

Intended Use

The PAXgene Blood ccfDNA System consists of a blood collection tube (PAXgene Blood ccfDNA Tube) and a ccfDNA purification kit (QIAsymphony PAXgene Blood ccfDNA Kit) for the QIAsymphony SP. The PAXgene Blood ccfDNA System is intended for the collection, storage and transport of blood, the stabilization of ccfDNA in a closed tube, and the subsequent isolation and purification of ccfDNA from plasma for research applications.

The PAXgene Blood ccfDNA Tube is a plastic, closed, evacuated tube intended for the collection, anticoagulation, transportation and storage of human whole blood specimens and the stabilization of ccfDNA. Using the tube in conjunction with the QIAsymphony PAXgene Blood ccfDNA Kit or the QIAamp® Circulating Nucleic Acid technology enables isolation of ccfDNA suitable for demanding downstream analytical assays.

The QIAsymphony PAXgene Blood ccfDNA Kit utilizes magnetic-particle technology for automated isolation and purification of ccfDNA from plasma, generated from specimens collected in PAXgene Blood ccfDNA Tubes.

The system performance has been established in studies in which ccfDNA is purified from human plasma generated from specimens collected in PAXgene Blood ccfDNA Tubes. Users must validate the performance of the system in their laboratory for specific research applications.

For research use only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Product Warranty and Satisfaction Guarantee

PreAnalytiX guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for particular uses. Should any product fail to perform satisfactorily due to any reason other than misuse, PreAnalytiX will replace it

free of charge or refund the purchase price. We reserve the right to change, alter or modify any product to enhance its performance and design. If a PreAnalytiX product does not meet your expectations, simply call your local QIAGEN Technical Service Department or PreAnalytiX distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to scientific instruments, service product and to products shipped on dry ice. Please inquire for more information.

A copy of PreAnalytiX terms and conditions can be obtained on request and is also provided on the back of our invoices. If you have questions about product specifications or performance, please contact PreAnalytiX Technical Services or your local distributor (see page 31 or visit **www.preanalytix.com**).

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIAsymphony PAXgene Blood ccfDNA 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 ccfDNA 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**).

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.preanalytix.com/resources where you can find, view and print the SDS for each PreAnalytiX kit and kit component.

If liquid containing buffers from the reagent cartridges 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.

Waste from plasma generation or ccfDNA isolation is to be considered potentially infectious. Dispose of potentially infectious waste according to your institution's waste-disposal guidelines.

Introduction

QIAsymphony 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.

The QIAsymphony PAXgene Blood ccfDNA Kit allows automated, standardized purification on the QIAsymphony SP of ccfDNA from 2.4 ml or 4.8 ml human plasma generated from human whole blood collected into PAXgene Blood ccfDNA Tubes. Proven magnetic-particle technology provides ccfDNA that is suitable for direct use in downstream applications. The QIAsymphony SP performs all steps of the sample preparation procedure. Up to 96 samples, in 4 batches of 24, can be processed in a single run.

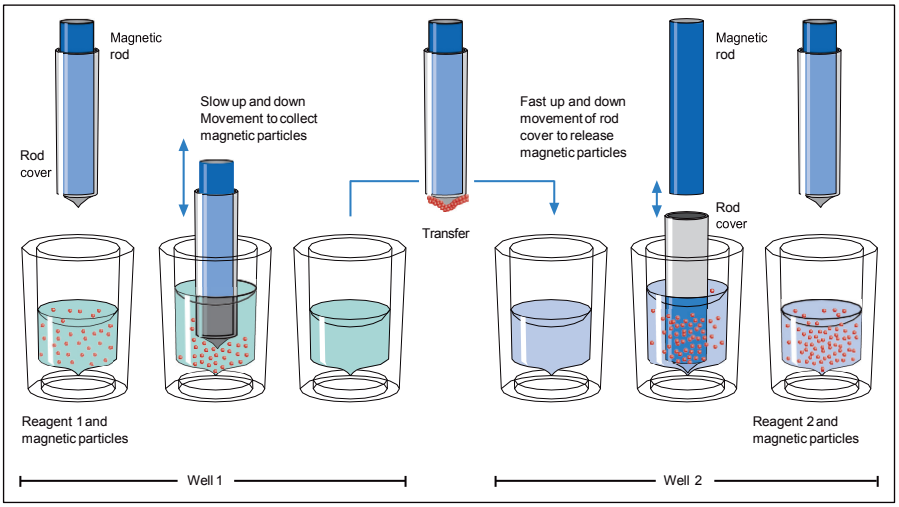

Figure 1. Schematic of the QIAsymphony SP principle. The QIAsymphony SP processes a sample containing magnetic particles as follows. A magnetic rod protected by a rod cover enters a well containing the sample and attracts the magnetic particles. The magnetic rod cover is positioned above another well 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 workflow begins with a centrifugation step to separate the plasma from the cellular fraction of human whole blood collected in PAXgene Blood ccfDNA Tubes. The plasma is transferred carefully into a secondary tube and (optional) a second centrifugation step is carried out. The pure plasma is transferred in a third tube and this tube is loaded on the QIAsymphony SP.

During a first processing step plasma proteins are digested by proteinase K while the ccfDNA binds to the surface of magnetic particles. Depending on the chosen protocol line (Figure 2) predominately small ccfDNA fragments or small and large ccfDNA fragments are isolated. Three wash steps guarantee that contaminants are removed. Finally, ccfDNA is diluted from the magnetic particles and is ready to use in downstream applications.

Purified ccfDNA can be used in downstream applications including but not limited to PCR and next generation sequencing (NGS) applications.

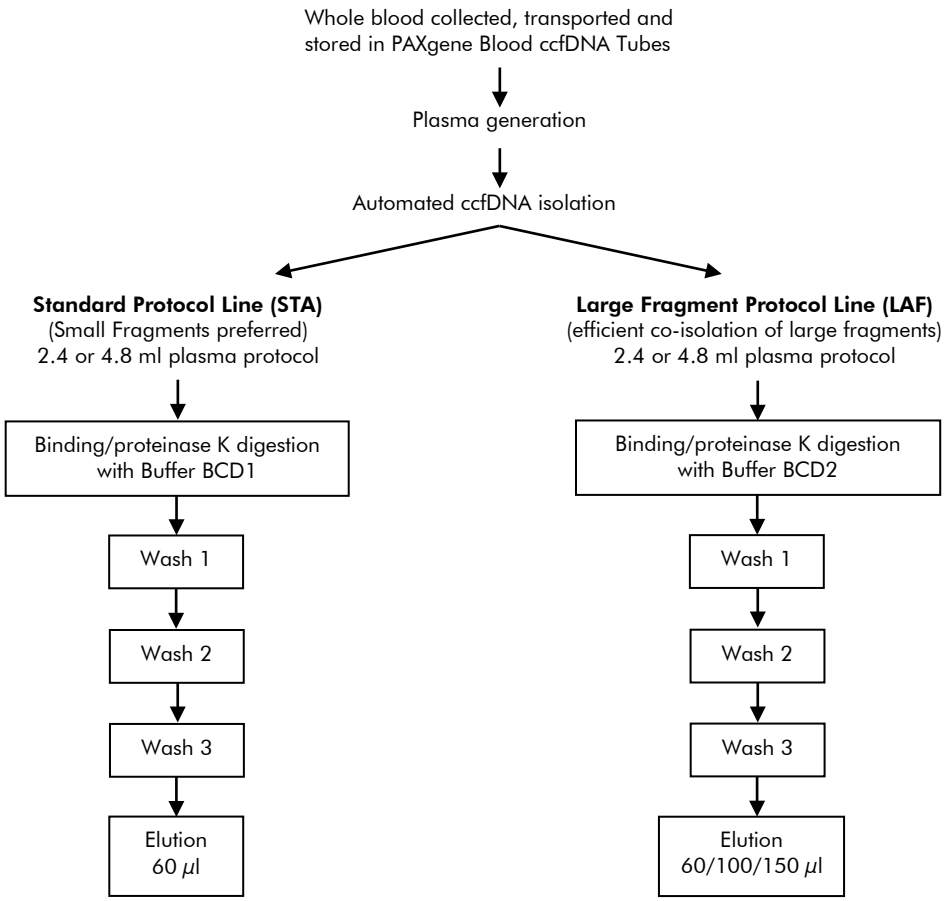

Figure 2. Standard and large fragment protocol lines. With the standard protocols (STA) predominately small ccfDNA fragments are isolated while large fragment protocols (LAF) efficiently co-purify large and small ccfDNA fragments. Both protocol lines consist of one protocol for 2.4 ml and one protocol for 4.8 ml plasma input.

Equipment and Reagents to Be Supplied by User

Consumables

- 8-Rod Covers (cat. no. 997004)
- Sample Prep Cartridges, 8-well (cat. no. 997002)
- Filter-Tips, 200 µl (cat. no. 990332) and 1500 µl (cat. no. 997024)
- Sterile, aerosol-barrier, RNase-free pipet tips
- 14 ml Falcon® polystyrene round-bottom tube, 17 × 100 mm (Corning, 352051)
- PAXgene Blood ccfDNA Tubes (cat. no. 768115)
- Tip Disposal Bags (cat. no. 9013395)

Equipment

- QIAsymphony SP* (cat. no. 9001297)
- Optional: QIAsymphony Cabinet SP* (cat. no. 9020244)
- Pipets* (200 µl – 5 ml)
- Centrifuge* capable of attaining at least 1900 × *g* and equipped with a swing-out (optional) rotor and buckets to hold PAXgene Blood ccfDNA Tubes
- Vortexer

Elution into Elution Microtubes CL (provided with the kit)

Equipment

- Spatula to remove the lower plate from the elution microtube rack
- Cooling Adapter, EMT, v2, Qsym

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

Elution into 1.5 ml Eppendorf® DNA LoBind Tube or 1.5 ml Sarstedt® tubes (not provided)

Consumables

- 1.5 ml DNA LoBind Tube, Snap Cap (Eppendorf, cat. no. 0030108.051)
- Micro tube 1.5ml, PP, NON-SKIRTED (Sarstedt, cat. no. 72.607) with Screw cap, neutral (Sarstedt, cat. no. 65.716.725)

Equipment

- Cooling Adapter, Snap-Cap Microtube QIAsymphony, Qsym
- Cooling Adapter, 2 ml, v2, Qsym

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

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 ccfDNA 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.

The complete PAXgene Blood ccfDNA workflow is described in "Protocol: Purification of ccfDNA from Human Plasma Generated from Human Whole Blood Collected into PAXgene Blood ccfDNA Tubes", page 19.

"Sample" drawer

Samples are loaded via the tube carriers into the "Sample" drawer. Plasma has to be filled into secondary sample tubes and specific labware files have to be selected on the user interface of the QIAsymphony SP (specified in Tables 1 and 2).

Additionally, proteinase K is loaded via the sample tube carrier in slot A, because this enzyme is not part of the Reagent Cartridges but is provided in glass vials with the kit. The amounts of proteinase K which has to be placed on the instrument is shown in Table 3.

Table 1. Sample drawer setup for purification of ccfDNA from plasma

Sample type	Human plasma generated from blood collected in PAXgene ccfDNA Tubes
Sample input volume	2.4 ml* (PAXcircDNA_STA_2400 or PAXcircDNA_LAF_2400) or 4.8 ml* (PAXcircDNA_STA_4800 or PAXcircDNA_LAF_4800)
Secondary sample tubes	14 ml, 17 × 100 mm polystyrene, round-bottom tubes (Corning, cat. no. 352051)
Inserts	n/a
Other	Proteinase K required in 14 ml, 17 × 100 mm polystyrene, round-bottom tubes (Corning, cat. no. 352051); only use positions 1 and 2 of the tube carrier (for slot A)

* For detailed information see “Sample volume”, page 11.

n/a = not applicable.

Sample volume

To transfer the complete 2.4 ml (PAXcircDNA_STA_2400 or FT_PAXcircDNA_LAF_2400) and 4.8 ml (PAXcircDNA_STA_4800 or PAXcircDNA_LAF_4800) sample during the isolation procedure, the QIAsymphony SP requires an additional 0.4 ml (2.8 ml total) and 0.5 ml (5.3 ml total) of sample, respectively, to account for the void volume that is not transferred to the isolation procedure. If less volume is provided, samples are flagged as “unclear” but are still processed. If less than 1800 µl sample are provided for the PAXcircDNA_STA_2400 or PAXcircDNA_LAF_2400 protocol, or less than 4200 µl for the PAXcircDNA_STA_4800 or PAXcircDNA_LAF_4800 protocol, samples are flagged as “invalid” and are not processed.

Important: With the sample tube format “BD_FIX #352051 FalconPP 17 × 100,” the QIAsymphony does not perform liquid level detection before aspiration of the sample. In this case, a void volume of 0.1 ml is required to

make sure that 2.4 ml sample for the PAXcircDNA_STA_2400 or PAXcircDNA_LAF_2400 protocols and 4.8 ml for the PAXcircDNA_STA_4800 or PAXcircDNA_LAF_4800 protocols are transferred. Less than 2.5 ml or 4.9 ml, respectively, may cause foam to form during sample processing.

Table 2. Sample tubes for tube carrier

Name on touchscreen	Supplier (cat. no.)	Material	Sample volume PAXcircDNA STA 2400 PAXcircDNA LAF 2400	Sample volume PAXcircDNA STA 4800 PAXcircDNA LAF 4800
BD #352051 FalconPP 17 × 100	Corning* (352051)	14 ml Falcon polystyrene round-bottom rube 17 × 100 mm	2.8 ml† 1.8 ml†§	5.3 ml† 4.2 ml†§
BD_FIX #352051 FalconPP 17× 100	Corning* (352051)	14 ml Falcon polystyrene round-bottom tube 17 × 100 mm	2.5 ml‡¶	4.9 ml‡¶

* Previously supplied by BD™.

† Minimum sample volume required per sample per protocol (including void volume); clot detection possible.

‡ Minimum sample volume required per sample per protocol (including void volume); clot detection not possible.

§ Reduced minimum sample volume. Make sure to use the "enable less sample" mode, which uses all available liquid in combination with liquid level detection and clot detection. The "enable less sample" mode results in "unclear" flagging of samples.

¶ Reduced minimum sample volume (ml) to minimize dead volumes. FIX labware is designed for this purpose and does not support liquid level detection or clot detection. FIX sample tubes impose aspiration restriction. Sample is aspirated at a defined height in the tube. This height is specified by the volume of sample to be transferred. Therefore, it is essential to make sure to use the volume listed in the table.

Table 3. Preparation of proteinase K in position 1 (and if required, in position 2) of slot A

Sample number	PAXcircDNA STA 2400*	PAXcircDNA STA 4800*
8	1980 µl	2860 µl
24	3740 µl	6380 µl
96	11,660 µl	11,660 µl†
	PAXcircDNA LAF 2400†	**PAXcircDNA LAF 4800†**
8	2060 µl	3020 µl
24	3980 µl	6860 µl
96	11,660 µl‡ (tube1) & 2060 µl (tube 2)	11,660 µl‡ (tube 1) & 11,660 µl (tube 2) & 3020 µl (tube 3)

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

† For each sample, 120 µl for PAXcircDNA LAF 2400 or 240 µl for PAXcircDNA LAF 4800, are required, plus an additional void volume of 1100 µl [(n × 120 or 240 µl) + 1100 µl].

‡ If more than 11,660 µl are required, use additional tubes (Corning, cat. no. 352051). An additional void volume of 1100 µl is required for each additional tube used.

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

"Reagents and Consumables" drawer

Reagent cartridges

Reagents for purification of ccfDNA from human plasma generated from whole blood collected in PAXgene Blood ccfDNA Tubes are contained in an innovative reagent cartridge (Figure 3). Only proteinase K has to be loaded in 14 ml Falcon tubes on slot A via a tube carrier (see "Sample" drawer, page 10). Each trough of the reagent cartridge contains a particular reagent, such as magnetic particles, binding buffers, wash buffers, or elution buffers. 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.

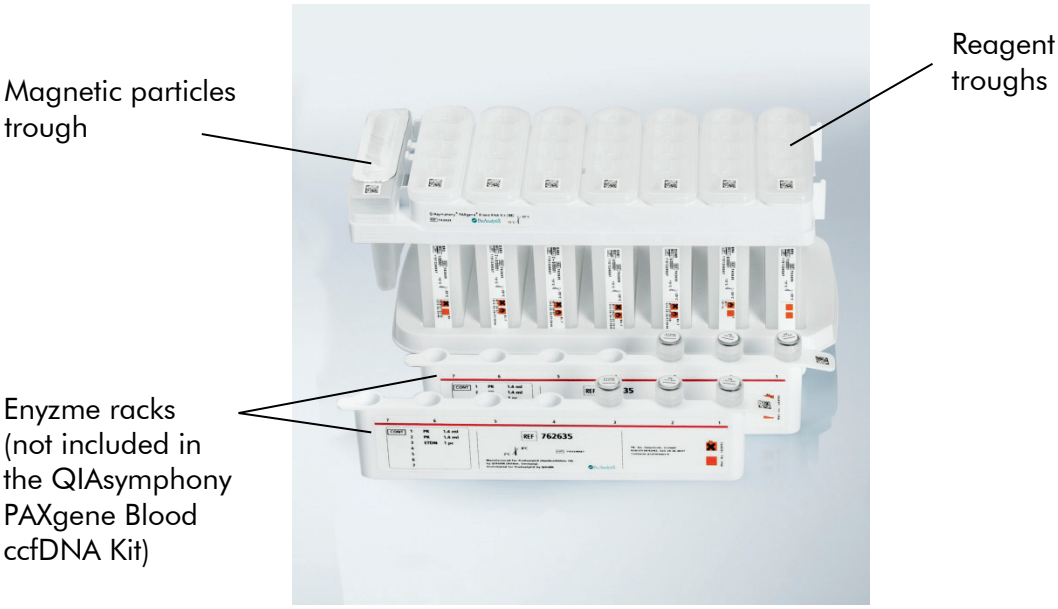

Figure 3. Reagent cartridge. The reagent cartridge contains all reagents required for the protocol run.

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. Place the piercing lid on top of the reagent cartridge (Figure 4).

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 4. Easy worktable setup with reagent cartridges. The enzyme rack is not included in the QIAsymphony PAXgene Blood ccfDNA Kit.

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; not provided) are loaded into the "Reagents and Consumables" drawer (see Table 4 and Table 7, page 18 for the consumables required).

For plasticware ordering information, see page 27.

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.

Table 4. Reagent and Consumables drawer setup for purification of ccfDNA

Position A1 and/or A2	Reagent cartridge
Position B1	n/a
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

n/a = not applicable.

"Waste" drawer

Sample prep cartridges and 8-Rod Covers used during a run are re-racked in empty unit boxes in the "Waste" drawer (Table 5). Make sure 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. Make sure that the tip disposal bag is properly attached before starting a protocol run. For more information, 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.

Table 5. Waste drawer setup for purification of ccfDNA from plasma

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

"Eluate" drawer

The required elution format is loaded into the "Eluate" drawer (see Table 6). 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.

Table 6. Eluate drawer setup for purification of ccfDNA from plasma

Supplier (cat. no)	Material	Category	Name on touchscreen	Adapter on Elution slot 1 (cooled)
QIAGEN (supplied with kit; 19588)	Elution Microtube CL 96	Deep Well	QIA#19588 *EMTR	Cooling Adapter, EMT, v2, Qsym
Eppendorf (0030108.051)	1.5 ml DNA LoBind Tube	Tube,1.5 ml	EP#0030108.051**T1.5 Snap Cap	Cooling Adapter, Snap-Cap Microtube QIAsymphony, Qsym
Sarstedt (72.607)	1.5 ml Micro tube, PP, NON-SKIRTED	Tube, 1.5 ml / Tube, 1.5 ml Adapter V1 (no BC)	SAR#72.607 *T1.5 Screw / SAR#72.607 **T1.5 Screw	Cooling Adapter, 2 ml, v2, Qsym

* Indicates labware that can be cooled using a cooling adapter with bar code (transferable and usable on QIAsymphony AS).

** Indicates labware that can be cooled using a cooling adapter without bar code (non-transferable and not usable on QIAsymphony AS).

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 7).

Table 7. Consumables required for purification of ccfDNA from plasma

	PAXcircDNA 2400 STA PAXcircDNA 2400 LAF		
Number of samples	**24**	**48**	**96**
Reagent cartridges*	1	1	2
Sample prep cartridges*	15	30	60
8-Rod Covers†	3	6	12
1500 µl tips‡	64	128	256
200 µl tips‡	24	48	96
	PAXcircDNA 4800 STA PAXcircDNA 4800 LAF		
Number of samples	**24**	**48**	**96**
Reagent cartridges*	1	1	2
Sample prep cartridges*	18	36	72
8-Rod Covers†	3	6	12
1500 µl tips‡	104	200	392
200 µl tips‡	24	48	96

* There are 28 sample prep cartridges/unit box.

† There are twelve 8-Rod Covers/unit box.

‡ There are 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 ccfDNA from Human Plasma Generated from Human Whole Blood Collected into PAXgene Blood ccfDNA Tubes

Important points before starting

- 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.
- Blood must be collected in PAXgene Blood ccfDNA Tubes (cat. no. 768115). For handling of these tubes and blood collection instructions see the PAXgene Blood ccfDNA Tube Product Circular provided with the PAXgene Blood ccfDNA Tubes.
- All steps of the QIAsymphony PAXgene Blood ccfDNA protocol for purification of ccfDNA from plasma should be performed at 15–25°C.

Things to do before starting

- Blood samples filled in PAXgene Blood ccfDNA Tubes are stable at room temperature (15–25°C) for up to 7 days or at higher temperatures (up to 35°C) for up to 1 day until centrifugation and plasma processing.
- 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.

Preparation of sample material

Plasma preparation from blood collected in PAXgene Blood ccfDNA Tubes:

1. **Centrifuge the PAXgene Blood ccfDNA Tube at room temperature (15–25°C) for 15 minutes at 1900 × *g* using a balanced centrifuge.**
   Note: For optimal separation of plasma, use a swing-out rotor centrifuge.
2. **Optional: For research applications that require further purification of the plasma, a second centrifuge step can be performed.**

2a. Pipet the plasma into a 15 ml conical bottom centrifugation tube (not provided). Take care not to disturb the buffy coat and the cellular fraction.

2b. Centrifuge the conical bottom centrifuge tube at room temperature (15–25°C) for 10 minutes at 1900 × *g* using a balanced centrifuge.

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

3. **Pipet the appropriate plasma volume (see "Sample volume", page 11) into a 14 ml, 17 × 100 mm polystyrene, round-bottom tubes (Corning, cat. no. 352051). Take care not to disturb the residual blood cell pellet at the bottom of the tube, if present. Remaining plasma can be pipetted into a suitable tube.**

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

4. **Transfer the plasma sample in the round-bottom tube into the tube carrier and load the tube carrier into the sample input drawer. Alternatively, store plasma after collection and centrifugation at 2–8°C for up to 24 hours. For longer storage, we recommend freezing aliquots.**

Freezing and thawing plasma from whole blood collected in PAXgene Blood ccfDNA Tubes (if necessary for long-term storage):

1. **Stand the 14 ml, 17 × 100 mm polystyrene, round-bottom tube or other suitable tubes containing plasma specimens upright in a wire rack.**
2. **Store the tubes at −20°C or at −70°C to −80°C.**
3. **Thaw the plasma at room temperature (15–25°C).**

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

4. **If cryoprecipitates form in the plasma, vortex the tube for 30 seconds after thawing and use the sample for the QIAsymphony SP ccfDNA isolation procedure without further treatment. Transfer the plasma sample into the tube carrier and load the tube carrier in the sample input drawer.**

   Note: Do not centrifuge the plasma to remove cryoprecipitates, as they may contain ccfDNA.

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

Automated ccfDNA isolation on the QIAsymphony SP

1. **Ensure that the QIAsymphony SP is switched on.**
   The power switch is located at the bottom left corner of the QIAsymphony SP.
2. **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.**
3. **Load the required reagent cartridge and consumables (see Table 3, page 13; Table 4; page 15, and Table 7, page 18) into the "Sample" and "Reagents and Consumables" drawers and perform an inventory scan of the "Reagents and Consumables" drawer.**
4. **Load the required elution format 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".
5. **Load the samples (from section Preparation of sample material, step 4, page 20) into the "Sample" drawer.**
6. **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 #352051 FalconPP 17 × 100" or "BD_FIX #352051 FalconPP 17 × 100" from the "Tube Insert 00" sheet)
   - Protocol ("Assay Control Set") to be run. Choose the best suited protocol variant as described in Figure 2, page 8.
   - Output position and if required elution volume.

   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.
7. **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.

8. **After the QIAsymphony protocol finishes, remove the elution format containing the purified ccfDNA and seal 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 the "Close" button) before the run can be restarted.

   Result files are generated for each elution plate.

9. **If the Microtubes CL were used, put the bottom plate back onto the rack for storage.**
10. **Store the purified ccfDNA at –15°C to –30°C or at –65°C to –90°C.**

    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 application, 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.

11. **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.**
12. **Discard used sample tubes and waste according to your local safety regulations.**

    See page 6 for safety information.

13. **Clean the QIAsymphony SP.**

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

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

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) Overnight runs	To optimize throughput, the PAXgene Blood ccfDNA System supports processing samples overnight, since eluates are cooled on the QIAsymphony SP instrument at the end of a run. During long cooling periods, eluate volumes can differ slightly from volumes directly after the run, 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 h storage on the instrument.
b) Error message displayed in the touchscreen	If an error message is displayed during a protocol run, refer to "Troubleshooting" in the QIAsymphony SP User Manual.
c) Instrument issue during the run	If the run stops because of an instrument issue during a run, the samples can be rescued via specific sample rescue protocols combined with a modified manual ccfDNA isolation procedure based on the QIAamp Circulating Nucleic Acid Kit. For sample rescue-protocols, please contact Technical Services at **www.preanalytix.com** or call your local distributor.

Cryoprecipitates occur 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 minutes instead of room temperature. Do not thaw at low temperatures (e.g., 4°C). Vortex the tube for 30 seconds after thawing and use the sample for the QIAsymphony SP ccfDNA isolation procedure.
b) Low ccfDNA yield from plasma after removing cryoprecipitates	Do not centrifuge the plasma to remove cryoprecipitates, as they may contain ccfDNA.

Insufficient plasma 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 Product Circular*).
b) Hematocrit above specification	The QIAsymphony PAXgene Blood ccfDNA Kit is intended for purification of cell-fee circulating DNA from human whole blood with hematocrit of less than 51% (male) or less than 47% (female).
c) Low plasma yield after centrifugation	Longer storage or transport times can have an impact on plasma yields.

Stabilization of ccfDNA is impacted

a) Compromised relative amount of ccfDNA	Optimal temperature range for transport and storage of blood collected in PAXgene Blood ccfDNA Tubes is 15–25°C. Longer incubation at high (e.g., >35°C) or low (e.g., <4°C) temperatures can have a negative impact on the relative amount of ccfDNA. Carryover of white blood cells during plasma generation will lead to genomic DNA contamination in the eluates of the ccfDNA isolation procedure and dilution of the original ccfDNA. Therefore careful transfer of plasma without disturbing the buffy coat after centrifugation of the blood is essential. To avoid even minimal cell carryover, a second centrifugation step as described on page 19, step 2 could be helpful.

ccfDNA does not perform well 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) Insufficient amount of ccfDNA used in downstream application	Quantify the purified ccfDNA by suitable methods like capillary electrophoresis (e.g., QIAxcel®, Agilent® BioAnalyzer) or sensitive assays (QIAxpert®, Qubit®, qPCR).
c) 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 could be spun down and transferred to a clean tube.

	Comments and suggestions
Low ccfDNA yield	
a) Less than 10 ml blood collected into the PAXgene Blood ccfDNA Tube	Ensure that 10 ml blood is collected into the PAXgene Blood ccfDNA Tube (see the *PAXgene Blood ccfDNA Tube Product Circular*). Together with 1.5 ml of cell stabilization additive a tube completely filled should contain a total volume of blood and additive of 11.5 ml.
b) Insufficient plasma processed by the instrument	Ensure that sufficient amount of plasma is loaded on the instrument. The void volumes needed depend on the chosen protocol and the sample input format used (see Table 1 on page 11 and Table 2 on page 12)
c) 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.
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 ccfDNA purification.

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.

Ordering information

Product	Contents	Cat. no.
QIAsymphony PAXgene Blood ccfDNA Kit (192)	For 192 preps: 2 Reagent Cartridges, Accessories and Proteinase K vials	768536
PAXgene Blood ccfDNA Tubes (100)	100 Tubes: 16 × 100 mm, 1.5 ml Additive, 10 ml Blood Draw Volume	768115
QIAsymphony SP	QIAsymphony sample prep module, 1 year warranty on parts and labor	9001297
Accessories		
Cooling Adapter, EMT, v2, Qsym	Cooling adapter for EMT racks; for use with the QIAsymphony SP/AS instruments (software version 3.1 or higher)	9020730
Cooling Adapter, 2 ml, v2, Qsym	Cooling adapter for 1.5 and 2 ml screw-cap tubes; for use with the QIAsymphony SP/AS instruments	9020674
Cooling Adapter, Snap-Cap Microtube QIAsymphony, Qsym	Adapter for holding Snap-Cap tubes	9020731
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 × 128)	997024
Filter-Tips, 200 µl (1024)	Sterile, Disposable Filter-Tips, racked; (8 × 128)	990332
Elution Microtubes CL (24 × 96)	Nonsterile polypropylene tubes; 2304 in racks of 96; includes cap strips	19588

Product	Contents	Cat. no.
Caps for Elution Microtubes (50x8)	Caps for Elution Microtubes (50x8)	19591
Tip Disposal Bags (15)	Tip disposal bags for use with the QIAsymphony SP/AS instruments	9013395
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
Related products		
QIAamp Circulating Nucleic Acid Kit (50)	QIAamp Mini Columns, Tube Extenders (20 ml), QIAGEN Proteinase K, Carrier RNA, Buffers, VacConnectors and Collection Tubes (1.5 ml and 2 ml)	55114
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
Related products that can be ordered from BD*		
Blood Collection Set	BD Vacutainer® Safety-Lok™ 6 Blood Collection Set: 21G, 0.75 inch needle, 12 inch tubing with luer adapter; 50 per box, 200 per case	367286
BD Vacutainer One-Use Holder	Case only for 13 mm and 16 mm diameter; 1000/case	364815

* These blood collection accessories represent typical products that can be used with PAXgene Blood ccfDNA Tubes. To find out more about these accessories, including how to order, visit **www.preanalytix.com**.

For up-to-date licensing information and product-specific disclaimers, see the respective PreAnalytiX 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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1104403 HB-2101-002 08/2016