

July 2023

QIASymphony® SP Instrument Instructions for Use (Protocol Sheet)

For the PreAnalytiX QIASymphony PAXgene® Blood ccfDNA Kit and PreAnalytiX PAXgene Blood ccfDNA Tube

PAXgene Blood ccfDNA IVD protocols:

PAXcircDNA _2400, PAXcircDNA _4800, PAXcircDNA_PrimaryTube_2400, and PAXcircDNA_PrimaryTube_4000

Version 1

IVD

For In Vitro Diagnostic Use



REF

768566



PreAnalytiX GmbH
Garstligweg 8, 8634 Hombrechtikon, Switzerland

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PreAnalytiX GmbH, 8634 Hombrechtikon, CH.

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General information

For in vitro diagnostic use.

The QIASymphony PAXgene Blood ccfDNA Kit 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 purification procedure is optimized for use with plasma generated from human venous whole blood collected in the PAXgene Blood ccfDNA Tubes. For instructions on the blood collection procedure, see the PAXgene Blood ccfDNA Tube Instructions for Use on the product homepage (www.preanalytix.com).

Four different protocols were established for automated isolation of ccfDNA from plasma generated from human venous whole blood collected in the PAXgene Blood ccfDNA Tubes. In the standard versions, sample input volumes of 2.4 or 4.8 mL plasma can be selected. In addition, primary tube handling protocols allow direct placement of the PAXgene Blood ccfDNA Tube onto the QIASymphony SP instrument. Primary tube handling protocols are available for sample input volumes of 2.4 or 4.0 mL plasma (see the tables in the next pages).

Each plasma volume used for ccfDNA extraction requires the relevant sample input volume including void volume and the relevant protocol script as summarized in Table 1.

Table 1. Protocol overview

Sample material	Human plasma generated from venous whole blood collected into PAXgene Blood ccfDNA Tubes			
Kit	QIASymphony PAXgene Blood ccfDNA Kit (CE-IVD)(192), cat. no. 768566			
Software version	Version 5.0 or higher			
Software configuration for IVD use	Default profile 1			
Protocols	Protocol line	Sample input volume (incl. void volume) (mL)	Sample volume used for ccfDNA extraction (mL)	(Assay Control_) Protocol name
	Standard	2.8 5.3	2.4 4.8	(ACS_)PAXcircDNA_2400 (ACS_)PAXcircDNA_4800
	Primary tube handling	according to selection tool	2.4 4.0	(ACS_)PAXcircDNA PrimaryTube_2400 (ACS_)PAXcircDNA PrimaryTube_4000

Plasma preparation can be done using (A) the standard double-centrifugation protocol or (B) via primary tube handling: direct processing of the one-time single 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 × 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.

2. Pipette 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 for 10 min at room temperature (15–25°C) at 1600–3000 × g using a balanced centrifuge.

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

4. Pipette the required plasma volume (see section “Sample volume” on page 9) into a 14 mL, 17 × 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 QIASymphony 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.

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 $3000 \times g$ 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.

2. 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 1). 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 QIAasymphony SP instrument

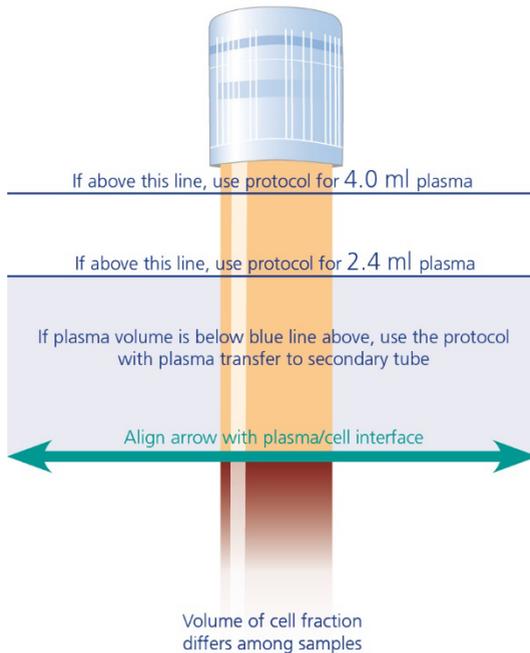


Figure 1. Plasma volume determination using the PAXgene Blood ccfDNA Purification Protocol Selection Tool (This is for illustration purpose only; please do not print because the actual size differs – not for use with samples).

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.

3. Remove the Hemogard Closure Cap from the PAXgene Blood ccfDNA Tubes before placement on the QIAasymphony SP instrument for direct ccfDNA extraction.
4. 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 QIAasymphony SP instrument.

Sample volume

To make sure that in the regular workflow 2.4 mL (PAXcircDNA_2400 protocol) and 4.8 mL samples (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. In case lower plasma volumes than the 2.8 or 5.3 mL are available, the **Less Sample mode** as integrated part of the protocol function 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 mL (PAXcircDNA 2400 protocol) and 4.1 mL (PAXcircDNA 4800 protocol). 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 and described section "B) Plasma preparation from blood for primary tube handling on the QIASymphony SP instrument" on page 6.

“Sample” drawer

Table 2. Information for sample drawer set-up*

Sample type	Human plasma generated from venous whole blood collected into the PAXgene Blood ccfDNA Tubes
Sample input volume (incl. void volume)	2.8 mL (PAXcircDNA_2400); 5.3 mL (PAXcircDNA_4800) See PAXgene Blood ccfDNA Purification Protocol Selection Tool (PAXcircDNA_PrimaryTube_2400) See PAXgene Blood ccfDNA Purification Protocol Selection Tool (PAXcircDNA_PrimaryTube_4000)
Primary sample tubes	10 mL PAXgene Blood ccfDNA Tube (CE-IVD) 16 × 100 mm (BD™, cat. no. 768165)
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)

* See also Labware list available under the Product Resources tab on www.qiagen.com or the Resources tab on www.prealanalytix.com.

n/a, not applicable.

Sample tubes for tube carrier

Table 3. Information for tube carrier set-up*

Name in touchscreen	Supplier	Material	Example cat. no.	Insert	PAXcirc DNA_2400	PAXcirc DNA_4800	PAXcircDNA_PrimaryTube_2400	PAXcircDNA_PrimaryTube_4000
BD #352051 FalconPP 17 × 100	Corning [†]	14 mL Falcon® polystyrene round-bottom tube 17 × 100 mm	352051	No insert needed	2.8 mL [‡] 1.6 mL [§] (Enable Less Sample mode)	5.3 mL [‡] 4.1 mL [§] (Enable Less Sample mode)	n/a	n/a
BD #768165 PAXgene ccfDNA 16 × 100	BD	10 mL PAXgene Blood ccfDNA Tube 16 × 100 mm	768165	No insert needed	n/a	n/a	See PAXgene Blood ccfDNA Purification Protocol Selection Tool	

* See also Labware list available under the Product Resources tab on www.qiagen.com or the Resources tab on www.preanalytix.com.

[†] Previously supplied by BD.

[‡] Minimum sample volume required per sample per protocol (including void volume); clot detection possible.

[§] Reduced minimum sample volume using **Enable Less Sample mode**. **Enable Less Sample mode** has been designed to use all available liquid in combination with liquid-level detection and clot detection. **Enable Less Sample mode** results in **unclear** flagging of samples.

n/a, not applicable.

“Reagents and Consumables” drawer

Position A1 and/or A2	Reagent cartridge
Position B1	n/a
Tip rack holder 1–17	Disposable filter-tips, 200 or 1500 µL
Unit box holder 1–4	Unit boxes containing sample prep cartridges or 8-Rod Covers

n/a = not applicable.

“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

Supplier	Material	Example cat. no.	Category	Name in touchscreen	Adapter on Elution slot 1 (cooled)
QIAGEN	Elution Microtubes CL 96	Supplied with the kit (19588)	Deep Well	QIA#19588* EMTR	Elution Microtube Rack QS
Eppendorf®	1.5 mL DNA LoBind® Tube	0030108.051	Tube, 1.5 mL	EP#0030108.051** T1.5 Snap Cap	Snap-Cap Microtube
Sarstedt®	1.5 mL Microtube, PP, non-skirted	72607	Tube, 1.5 mL / Tube, 1.5 mL Adapter V1 (no BC)	SAR#72.607* T1.5 Screw/SAR#72.607** T1.5 Screw	Microtube Screw Cap QS
Sarstedt	2.0 mL Microtube, PP, non-skirted	72693	Tube 2.0 mL / Tube_2.0 mL AdapterV1 (no BC)	SAR#72.693 *T2.0 Screw	Microtube Screw Cap QS
Starlab®	1.5 mL Microtube, graduated conical tube, non-skirted	E1415-2231	Tube, 1.5 mL / Tube_1.5 mL AdapterV1 (no BC)	SL#E1415-2231 *T1.5 Screw	Microtube Screw Cap QS, 24-wells, cat. no. 9020674 (cooling slot 1)
				SL#E1415-2231 **T1.5 Screw	Microtube Screw Cap QS (cooling slot 1)
				SL#E1415-2231 T1.5 Screw	1.5/2.0 mL QS (noncooling slots 2-4)

* 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).

Required plasticware

Plasticware	PAXcircDNA_2400		PAXcircDNA_4800	
	PAXcircDNA PrimaryTube_2400		PAXcircDNA PrimaryTube_4000	
	One batch, 24 samples*	Two batches, 48 samples*	One batch, 24 samples*	Two batches, 48 samples*
Disposable filter-tips, 200 µL [†]	24	48	24	48
Disposable filter-tips, 1500 µL [†]	64	128	104	200
Sample prep cartridges [§]	15	30	18	36
8-Rod Covers [¶]	3	6	3	6
	Three batches, 72 samples*	Four batches, 96 samples*	Three batches, 72 samples*	Four batches, 96 samples*
Disposable filter-tips, 200 µL [†]	72	96	72	96
Disposable filter-tips, 1500 µL [†]	192	256	296	392
Sample prep cartridges [§]	45	60	54	72
8-Rod Covers [¶]	9	12	9	12

* Using less than 24 samples per batch decreases the number of disposable filter-tips required per run. Performing more than one inventory scan requires additional disposable filter-tips.

[†] There are 32 filter-tips/filter-tip rack.

[‡] Number of required filter-tips includes filter-tips for 1 inventory scan per reagent cartridge.

[§] There are 28 sample prep cartridges/unit box.

[¶] There are twelve 8-Rod Covers/unit box.

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

Elution volume

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.

Preparation of Proteinase K in position 1 (and if required, in position 2) of slot A

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	11,660†
96	11,660†	23,320†

* 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 × 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 × 100 mm polystyrene, round-bottom tubes (Corning, cat. no. 352051) for Proteinase K.

Document Revision History

Date	Changes
04/2021	Initial release
05/2022	Removed "(CE-IVD)" in some occurrences. Updated one of the notes on protocol step 5 under section "A) Plasma preparation from blood for standard protocols". Updated section "Sample volume". Included www.preanalytix.com as to where related resources can be found.
07/2023	Street address of PreAnalytiX GmbH changed from "Feldbachstrasse" to "Garstligweg 8". Updated to new branding guidelines.



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