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QIAsymphony® SP Protocol Sheet

PAXgene® ccfDNA_2400 protocols (PAXcircDNA_STA_2400, PAXcircDNA_LAF_2400) and PAXgene ccfDNA_4800 protocols (PAXcircDNA_STA_4800, PAXcircDNA_LAF_4800)

QIAGEN

General information

These protocols are for isolation of circulating cell-free DNA (ccfDNA) in plasma generated from human whole blood collected in PAXgene Blood ccfDNA Tubes using the QIAsymphony SP and the QIAsymphony PAXgene Blood ccfDNA Kit.

Two protocol lines allow processing a starting sample volume of 2.4 ml or 4.8 ml plasma, depending on needs of planned downstream analyses. The standard protocol line (STA) preferentially purifies small ccfDNA fragments. The large fragment protocol line (LAF) provides an efficient co-isolation of large ccfDNA fragments in addition to an unbiased isolation of small ccfDNA fragments. Protocols of the LAF line also allow a choice of three different elution volumes. Purified DNA is ready for use in downstream applications or can be stored at –30°C to –15°C. More detailed information can be found in the QIAsymphony PAXgene Blood ccfDNA Kit Handbook and at **www.preanalytix.com**.

The QIAsymphony PAXgene Blood ccfDNA Kit must be used in accordance with the associated QIAsymphony PAXgene Blood ccfDNA protocols. It is your responsibility to use these materials in alignment with all applicable laws.

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

Note: Use the protocol script that corresponds to the sample volume to be processed.

Sample material	Human plasma generated from whole blood collected in PAXgene Blood ccfDNA Tubes			
Kit	QIAsymphony PAXgene Blood ccfDNA Kit (192) (cat. no. 768536)			
Required software version	Version 4.0 or higher			
Protocols	**Protocol line**	**Sample volume (ml)**	**Protocol name**	**Default Assay Control Set**
	Standard	2.4	PAXcircDNA_STA_2400	ACS_PAXcircDNA_STA_2400
		4.8	PAXcircDNA_STA_4800	ACS_PAXcircDNA_STA_4800
	Large fragment	2.4	PAXcircDNA_LAF_2400	ACS_PAXcircDNA_LAF_2400
		4.8	PAXcircDNA_LAF_4800	ACS_PAXcircDNA_LAF_4800
Editable parameters	Elution volume can be 60, 100 or 150 µl in the large fragment protocols			

Materials required but not provided

- 15 ml conical-bottom centrifugation tubes
- 14 ml, 17 × 100 mm polystyrene, round-bottom tubes (e.g., Corning®, cat. no. 352051)

Preparation of sample material

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.

This purification procedure is optimized for use with plasma generated from whole blood collected in PAXgene Blood ccfDNA Tubes. For instructions on handling of these tubes and collecting blood see the *PAXgene Blood ccfDNA Tube Product Circular*.

Preparing plasma from whole blood collected in PAXgene Blood ccfDNA Tubes

1. Centrifuge the PAXgene Blood ccfDNA Tube for 15 min at room temperature (15–25°C) and 1900 × *g* using a balanced centrifuge.

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

   Note: For optimal separation of plasma, use a swing-out rotor centrifuge.

2. **Optional**: For research applications that require further purification of the plasma, perform a second centrifugation. Otherwise, continue with step 3.

   Pipet the plasma into a 15 ml conical bottom centrifugation tube, making sure to not disrupt the buffy coat and the cellular fraction.

   Centrifuge for 10 min at room temperature (15–25°C) and 1900 × *g* using a balanced centrifuge.

3. Pipet the required plasma volume (see "Sample volume" section on page 4) into a 14 ml, 17 × 100 mm polystyrene, round-bottom tube. Do not disturb the residual blood cell pellet at the bottom of the tube, if present.

   Note: To maximize ccfDNA yield, process the largest allowable plasma volume.

   Note: After collection and centrifugation, plasma can be stored at 2–8°C for up to 24 h. For longer storage, we recommend freezing aliquots.

4. Place the sample tube into the tube carrier and load the tube carrier into the sample input drawer.

Freezing and thawing plasma from whole blood collected in PAXgene Blood ccfDNA Tubes

1. Place the polystyrene tubes containing plasma upright in a wire rack.
2. Store the rack of tubes with plasma at –20°C. To store at lower temperatures, freeze the tubes first at –20°C for 24 h and then transfer to –70°C or –80°C.
3. Thaw the tubes at room temperature (15–25°C) or 30 min at 30°C.

   Note: Do not thaw at lower 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 isolation procedure without further treatment.

   Note: Do not centrifuge plasma to remove cryoprecipitates because they may contain ccfDNA.

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

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 sample volume may result in a sample flagged as "invalid".

“Sample” drawer

Sample type	Human plasma from whole blood collected in PAXgene Blood 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	Not applicable
Other	Proteinase K required in 14 ml, 17 × 100 mm polystyrene, round-bottom tubes; use only positions 1 and 2 of the tube carrier (for slot A)

* For detailed information, see “Sample volume” section on page 4.

Sample tubes for tube carrier

Name on touchscreen	**Supplier/example cat. no.**	**Material**	**Insert**	**PAXcircDNA STA_2400** **PAXcircDNA LAF_2400**	**PAXcircDNA STA_4800** **PAXcircDNA LAF_4800**
BD #352051 FalconPP 17 × 100	Corning*/352051	14 ml Falcon® polystyrene round-bottom tube 17 × 100 mm	No insert needed	2.8 ml[†] 1.8 ml[†‡] (Enable less sample mode)	5.2 ml[†] 4.2 ml[†‡] (Enable less sample mode)
BD_FIX #352051 FalconPP 17 × 100	Corning*/352051	14 ml Falcon polystyrene round-bottom tube 17 × 100 mm	No insert needed	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 is reduced in Enable less sample mode, which was designed to use all available liquid in combination with Liquid Level Detection and clot detection. Samples are flagged as “unclear” but are still processed.

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

¶ Minimum sample volume is reduced to minimize dead volume. FIX labware is designed for this purpose and does not support Liquid Level Detection and clot detection. FIX sample tubes impose aspiration restrictions. Sample fluid is aspirated at a defined height in the tube, which is defined by the volume of sample to be transferred. Thus, it is essential to use the volume listed in the table.

"Reagents and Consumables" drawer

Position A1 and/or A2	Reagent cartridge
Position B1	Not applicable
Tip rack holder 1–17	Disposable filter-tips, 200 µl or 1500 µl
Unit box holder 1–4	Unit boxes containing sample prep cartridges or 8-Rod Covers

"Waste" drawer

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

"Eluate" drawer

Supplier	Material	Example cat. no.	Category	Name on 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 Micro tube, PP, NON-SKIRTED	72.607	Tube, 1.5 ml Tube, 1.5 ml Adapter V1 (no BC)	SAR#72.607*T1.5 Screw SAR#72.607 **T1.5 Screw	Micro Tube Screw Cap QS

* Labware can be cooled using a cooling adapter with bar code (can be transferred and used on the QIAsymphony AS)

** Labware can be cooled using a cooling adapter without bar code (cannot be transferred or used on the QIAsymphony AS)

Required plasticware

Plasticware	PAXcircDNA_STA_2400 PAXcircDNA_LAF_2400		PAXcircDNA_STA_4800 PAXcircDNA_LAF_4800	
	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
	One batch, 72 samples*	Two batches, 96 samples*	One batch, 72 samples*	Two 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 1 inventory scan requires additional disposable filter-tips.

† Number of required filter-tips includes 1 inventory scan per reagent cartridge. There are 32 filter-tips per filter-tip rack.

‡ There are 28 sample prep cartridges per unit box.

¶ There are twelve 8-Rod Covers per unit box.

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

Elution volume

Elution volume on touchscreen (µl)*	Required elution buffer volume (µl)†
60	75
100‡	105
150‡	155

* The elution volume selected on the touchscreen. 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. For 1.5 ml Eppendorf LoBind Tubes, the minimum accessible volume of eluate is 55 µl.

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

‡ Can only be selected in protocols of the large fragment protocol line (LAF).

Preparation of proteinase K in positions 1 and 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), but if storing for extended periods of time, we suggest keeping the enzyme vials with proteinase K at 2–8°C.

Tubes containing proteinase K are placed in a tube carrier. Place the tube carrier 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.

Sample number	PAXcircDNA_STA_2400*	PAXcircDNA_LAF_2400†	PAXcircDNA_STA_4800*	PAXcircDNA_LAF_4800†
8	1980 µl	2060 µl	2860 µl	3020 µl
24	3740 µl	3980 µl	6380 µl	6860 µl
48	6380 µl	6860 µl	11,660 µl‡	11,660 µl‡ (tube 1) + 2060 µl (tube 2)

* 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 a second tube (Corning, cat. no. 352051). For the second tube, an additional 1100 µl are required as void volume.

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