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QIAsymphony® SP Protocol Sheet

circDNA_2000_DSP_V1 and circDNA_4000_DSP_V1

This document is the QIAsymphony circDNA_2000_DSP_V1 and circDNA_4000_DSP_V1 Protocol Sheet, Version 1, R1

Sample to Insight

QIAGEN

General information

For in vitro diagnostic use.

This protocol is for purification of human circulating cell-free DNA from fresh or frozen human plasma and urine using the QIAsymphony SP and the QIAsymphony DSP Circulating DNA Kit.

Kit	QIAsymphony DSP Circulating DNA Kit (cat. no. 937556)
Sample material	Human plasma: EDTA or citrate anti-coagulated, or ccfDNA stabilized Human urine: non-stabilized or stabilized
Protocol name	circDNA_2000_DSP_V1 circDNA_4000_DSP_V1
Default Assay Control Set	ACS_circDNA_2000_DSP_V1 ACS_circDNA_4000_DSP_V1
Elution volume	60 µl
Required software version	Version 4.0.3 or higher

"Sample" drawer

Sample type	Human plasma (see "Preparation of sample material") and Human urine (stabilized or non-stabilized)
Sample volume	Depends on type of sample tube used For more information, see the labware list which can be found under the resource tab of the product page on **www.qiagen.com**.
Primary sample tubes	n/a
Secondary sample tubes	For more information, see the labware list which can be found under the resource tab of the product page on **www.qiagen.com**.
Inserts	n/a
Other	Proteinase K needs to be added in slot A (position 1 and/or 2)

n/a = not applicable.

Preparation of proteinase K in "Sample" drawer

The QIAsymphony DSP Circulating DNA Kit contains ready-to-use proteinase K solution that can be stored at room temperature.

Note: Tubes containing proteinase K are placed in a tube carrier. The tube(s) containing the proteinase K must be placed on positions 1 and/or 2 in slot A of the "Sample" drawer. For required tube type, see the labware list which can be found under the resource tab of the product page on **www.qiagen.com**.

Number of samples*	circDNA_2000_DSP	circDNA_4000_DSP
8	1980 µl	2860 µl
24	3740 µl	6380 µl
48	6380 µl	11.660 ml†
96	11.660 ml	22.220 ml†

* For each sample, 110 µl for circDNA_2000_DSP or 220 µl for circDNA_4000_DSP are required, plus an additional void volume of 1100 µl [(n x 110 or 220 µl) + 1100 µl].

† For circDNA_4000_DSP: If more than 48 samples are processed, use a second tube. The maximum loading volume per tube is 11.660 µl. For the second tube, an additional void volume of 1100 µl is required.

"Reagents and Consumables" drawer

Position A1 and/or A2	Reagent cartridge
Position B1	n/a
Tip rack holder 1–18	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

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

"Eluate" drawer

Elution rack (it is recommended to use slot 1, cooling position)	For more information, see the labware list which can be found under the resource tab of the product page on **www.qiagen.com**.

Required plasticware

Protocol circDNA_2000_DSP

Plasticware	One batch 24 samples*	Two batches 48 samples*	Four batches 96 samples*
Disposable filter-tips, 200 µl†‡	24	48	96
Disposable filter-tips, 1500 µl†‡	64	120	232
Sample prep cartridges§	15	30	60
8-Rod Covers¶	3	6	12

* Using less than 24 samples per batch decreases the number of disposable filter-tips required per run.

† 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.

Protocol circDNA_4000_DSP

Plasticware	One batch 24 samples*	Two batches 48 samples*	Four batches 96 samples*
Disposable filter-tips, 200 µl†‡	24	48	96
Disposable filter-tips, 1500 µl†‡	104	200	392
Sample prep cartridges§	18	36	72
8-Rod Covers¶	3	6	12

* Using less than 24 samples per batch decreases the number of disposable filter-tips required per run.

† 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: Numbers of filter-tips given may differ from the numbers displayed in the touchscreen depending on settings. It is recommended to load the maximum possible number of tips.

Elution volume

Selected elution volume	Initial elution volume
60 µl	75 µl

Elution volume is selected in the touchscreen. The mean available elution volume is ≥60 µl. In individual cases the final eluate volume for single samples may be up to 5 µl less than the selected volume (e.g., 55 µl). It is recommended to check the actual eluate volume when using an automated assay setup system which does not verify the eluate volume prior to transfer.

Storage of Eluates

It is recommended to remove the eluate plate from the "Eluate" drawer immediately after the run has finished. Elution plates may be left in the QIAsymphony SP after the run is completed overnight (maximum 16 hours including run time; recommended environmental conditions: 18–26°C and 20–75% relative humidity). Depending on temperature and humidity, eluate may experience condensation or evaporation.

After sample preparation, eluates can be stored at 2–8°C for up to 1 month. For long term storage, eluates can be stored at –20°C or at –80°C. Frozen eluates must not be thawed more than 3 times.

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.

Important points before starting

- Prevent formation of foam in or on the samples.
- Samples should be equilibrated to room temperature (15–25°C) before starting the run.

Human plasma

Blood samples treated with EDTA or citrate as anticoagulant can be used for plasma preparation. Plasma prepared from ccfDNA stabilized blood collection tubes can also be used. Plasma is generated as specified by the manufacturer.

It is recommended to perform plasma separation immediately after blood donation when using EDTA or citrate as anticoagulant.

For certain downstream applications it may be necessary to exclude or to minimize nucleic acids from vesicles. For such cases, it is recommended to perform a high-speed centrifugation step at 16,000 x *g* for 10 minutes at room temperature (15–25°C) after initial plasma generation.

After collection and centrifugation, plasma can be stored at room temperature for up to 7 days and at 2–8°C for up to 14 days. For longer storage, it is recommended to freeze aliquots at –20°C or –80°C. Frozen plasma must not be thawed more than 3 times. Repeated freeze–thawing leads to denaturation and precipitation of proteins, potentially resulting in reduced yields of circulating cell-free nucleic acids. If cryoprecipitates are visible in the samples, centrifuge at 6,800 x *g* for 3 minutes at room temperature (15–25°C) and transfer the supernatants without disturbing the pellets to a secondary sample tube (see the labware list which can be found under the resource tab of the product page on **www.qiagen.com**). Start the purification procedure immediately.

Human urine

Due to rapid degradation of circulating cell-free DNA after urine collection, it is strongly recommended to stabilize urine samples immediately.

Human urine stabilized

Stabilized urine may be stored at room temperature (15–25°C) or at 2–8°C for up to 7 days. For longer storage, it is recommended to freeze aliquots at –20°C or –80°C.

Stabilized urine samples require no sample pretreatment. After stabilization, it is recommended to centrifuge urine samples at low speed (1900 x *g*) for 10 minutes at room temperature (15–25°C) to remove cells prior to extraction of circulating cell-free DNA. If precipitates are visible in supernatants after centrifugation, warm the samples to 25°C in a water bath to dissolve precipitates. Before starting a run, transfer stabilized urine samples to a secondary sample tube then load this tube on the sample carrier (see the labware list which can be found under the resource tab of the product page on **www.qiagen.com)**.

Human urine "non-stabilized"

Before starting a protocol that requires Buffer ATL, check whether precipitate has formed in Buffer ATL. If necessary, dissolve by heating at 70°C with gentle agitation in a water bath. Aspirate bubbles from the surface of Buffer ATL.

Note: Buffer ATL (Buffer ATL, 4 x 50 ml, cat.-no. 939016) is not part of the QIAsymphony DSP Circulating DNA Kit and must be ordered separately.

It is recommended to centrifuge urine samples immediately after collection at low speed (1900 x *g*) for 10 minutes at room temperature (15–25°C) to remove cells. Non-stabilized urine samples require sample pretreatment.

Important: Equilibrate samples to room temperature (15–25°C) before starting pretreatment.

Important: Centrifugation and pretreatment should be performed within 4 hours of urine sample collection.

- Mix 2500 µl urine (circDNA_2000_DSP) or 4500 µl urine (circDNA_4000_DSP) with 250 µl or 450 µl Buffer ATL, respectively.
- Incubate the samples at room temperature (15–25°C) for 1 hour.
- Centrifuge samples at 1900 x *g* for 10 minutes at room temperature (15–25°C).

  If precipitates are visible in supernatant after centrifugation, warm the samples to 25°C in a water bath to dissolve precipitates.
- Transfer supernatants to a secondary sample tube then load this tube on the sample carrier (see the labware list which can be found under the resource tab of the product page on **www.qiagen.com**)

Important: Stability and integrity of circulating cell-free DNA is limited in non-stabilized urine. It is recommended to load a maximum of one batch of 24 samples per QIAsymphony run to minimize on-board time of urine samples.

Interfering substances

Plasma samples with high concentrations of gamma-globulin (>30 g/l) may lead to reduced recovery of circulating cell-free DNA.

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