June 2024

QIAsymphony® DSP Circulating DNA Kit Instructions for Use (Protocol Sheet)

circDNA_1000_DSP_V2, circDNA_2000_DSP_V4, circDNA_4000_DSP_V4,

circDNA_6000_DSP_V1, circDNA_8000_DSP_V1, circDNA_10000_DSP_V1

IVD

For In Vitro Diagnostic Use

For use with

	Σ	REF	Version
QIAsymphony DSP Circulating DNA Kit (192)	192	937556	V2
QIAsymphony DSP Circulating DNA Maxi Kit (192)	192	937566	V1
QIAsymphony DSP Circulating DNA Kit (96)	96	937555	V1

CE

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R3

The protocol sheet available electronically and can be found under the resource tab of the product page on **www.qiagen.com**.

Sample to Insight

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 DSP Circulating DNA Kit and the QIAsymphony SP instrument.

Kit	QIAsymphony DSP Circulating DNA Kit (192)		QIAsymphony DSP Circulating DNA Kit (96)
Catalog no.	937556		937555
Sample material	**Human plasma:** • From blood collection tubes with ccfDNA profile stabilizers • From blood collection tubes without ccfDNA profile stabilizers **Human urine:** • With cfDNA profile stabilizers • Without cfDNA profile stabilizers		
Protocol name	circDNA_1000_DSP_V2	circDNA_2000_DSP_V4	circDNA_4000_DSP_V4
Default Assay Control Set	ACS_circDNA_1000_DSP_V2	ACS_circDNA_2000_DSP_V4	ACS_circDNA_4000_DSP_V4
Elution Volume	60 µL	60 µL	60 µL
Required software version	Version 5.0 or higher	Version 5.0 or higher	Version 5.0 or higher
Required software configuration for IVD use	Default Profile 1	Default Profile 1	Default Profile 1

Kit	QIAsymphony DSP Circulating DNA Maxi Kit (192)		QIAsymphony DSP Circulating DNA Kit (96)
Catalog no.	937566		937555
Sample material	**Human plasma:** • From blood collection tubes with ccfDNA profile stabilizers • from blood collection tubes without ccfDNA profile stabilizers **Human urine:** • With cfDNA profile stabilizers • Without cfDNA profile stabilizers		
Protocol name	circDNA_6000_DSP_V1	circDNA_8000_DSP_V1	circDNA_10000_DSP_V1
Default Assay Control Set	ACS_circDNA_6000_DSP_V1	ACS_circDNA_8000_DSP_V1	ACS_circDNA_10000_DSP_V1
Elution Volume	60 µL	60 µL	60 µL
Required software version	Version 5.0 or higher	Version 5.0 or higher	Version 5.0 or higher
Required software configuration for IVD use	Default Profile 1	Default Profile 1	Default Profile 1

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.

"Sample" drawer

Sample type	Human plasma and urine (see "Preparation of sample material")
Sample volume	Depends on type of sample tube used For more information, see the labware list that can be found under the resource tab of the product page on **www.qiagen.com**.
Primary sample tubes	Not applicable
Secondary sample tubes	For more information, see the labware list that can be found under the resource tab of the product page on **www.qiagen.com**.
Inserts	Depends on type of sample tube used For more information, see the labware list that can be found under the resource tab of the product page on **www.qiagen.com**
Other	Proteinase K needs to be added in slot A (position 1, 2, and/or 3)

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.

Using the QIAsymphony DSP Circulating DNA Kit (96) (Catalog no. 937555) with 6 mL, 8 mL, and 10 mL sample input, additional Proteinase K (Catalog no.19134) has to be ordered to process 96 samples in total.

Additional Proteinase K bottles to be ordered to process 96 samples in total			
Protocol	circDNA_6000_DSP	circDNA_8000_DSP	circDNA_10000_DSP
Proteinase K bottle	1	2	3

Note: The number of required Proteinase K bottles depends on the batch size (refer to the table below for calculation of exact Proteinase K volume required).

Note: Tubes containing Proteinase K are placed in a tube carrier. The tube containing the Proteinase K must be placed preferentially on position 1. When several tubes must be loaded, it must be placed on position 1, 2, and/or 3 in slot A of the "Sample" drawer. For required tube type, see the labware list that can be found under the resource tab of the product page on **www.qiagen.com**.

Number of samples*	circDNA_1000_DSP (µL)	circDNA_2000_DSP (µL)	circDNA_4000_DSP (µL)	circDNA_6000_DSP (µL)	circDNA_8000_DSP (µL)	circDNA_10000_DSP (µL)
8	1580	1980	2860	3740	4620	5500
24	2540	3740	6380	9020	11,660	15,400§
48	3980	6380	11,660	18,040‡	23,320‡	29,700§
72	5420	9020	18,040†	27,060‡		
96	6860	11,660	23,320†			

* For each sample, 60 µL for circDNA_1000_DSP, 110 µL for circDNA_2000_DSP, or 220 µL for circDNA_4000_DSP, 330 µL for circDNA_6000_DSP, 440 µL for circDNA_8000_DSP or 550µl for circDNA_10000_DSP are required, plus an additional void volume of 1100 µL [(n x 60, 110, 220 µL, 330, 440 or 550 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.

‡ For circDNA_6000_DSP and circDNA_8000_DSP: If more than 24 samples are processed, use a second tube (up to 3 tubes can be used depending on sample number). The maximum loading volume per tube is 11.660 µL. For each tube, an additional void volume of 1100 µL is required.

§ For circDNA_10000_DSP: If more than 19 samples are processed, use a second tube (up to 3 tubes can be used depending on sample number). The maximum loading volume per tube is 11.660 µL. For each tube, an additional void volume of 1100 µL is required.

“Reagents and Consumables” drawer

Position A1 and/or A2	Reagent cartridge (RC)
Position B1	Not applicable
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

“Waste” drawer

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

“Eluate” drawer

Elution rack (we recommend using slot 1, cooling position)	For more information, see the labware list that can be found under the resource tab of the product page on **www.qiagen.com**.

Required plasticware

Protocol circDNA_1000_DSP

Plasticware	One batch 24 samples*	Two batches 48 samples*	Three batches 72 samples*	Four batches 96 samples*
Disposable filter-tips, 200 µL†‡	28	56	84	112
Disposable filter-tips, 1500 µL†‡	64	120	176	232
Sample prep cartridges§	15	30	45	60
8-Rod Covers¶	3	6	9	12

* Use of less than 24 samples per batch decreases the number of disposable filter-tips required per run.
† There are 32 filter-tips/tip rack.
‡ Number of required filter-tips includes filter-tips for 1 inventory scan per RC.
§ There are 28 sample prep cartridges/unit box.
¶ There are twelve 8-Rod Covers/unit box.

Protocol circDNA_2000_DSP

Plasticware	One batch 24 samples*	Two batches 48 samples*	Three batches 72 samples*	Four batches 96 samples*
Disposable filter-tips, 200 µL†‡	28	56	84	112
Disposable filter-tips, 1500 µL†‡	64	120	176	232
Sample prep cartridges§	15	30	45	60
8-Rod Covers¶	3	6	9	12

* Use of fewer than 24 samples per batch decreases the number of disposable filter-tips required per run.
† There are 32 filter-tips/tip rack.
‡ Number of required filter-tips includes filter-tips for 1 inventory scan per RC.
§ 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*	Three batches 72 samples*	Four batches 96 samples*
Disposable filter-tips, 200 µL†‡	28	56	84	112
Disposable filter-tips, 1500 µL†‡	104	200	298	394
Sample prep cartridges§	18	36	54	72
8-Rod Covers¶	3	6	9	12

* Use of fewer than 24 samples per batch decreases the number of disposable filter-tips required per run.
† There are 32 filter-tips/tip rack.
‡ Number of required filter-tips includes filter-tips for 1 inventory scan per RC.
§ There are 28 sample prep cartridges/unit box.
¶ There are twelve 8-Rod Covers/unit box.

Note: The required consumables limit the sample number for a complete run without hands-on time (only 18 Tip racks are available on deck layout) for the circDNA_6000_DSP, circDNA_8000_DSP, and circDNA_10000_DSP protocols.

Protocol circDNA_6000_DSP

Plasticware	One batch 24 samples*	Two batches 48 samples*	Three batches 72 samples*
Disposable filter-tips, 200 µL†‡	28	56	84
Disposable filter-tips, 1500 µL†‡	148	284	424
Sample prep cartridges§	21	42	63
8-Rod Covers¶	3	6	9

* Use of fewer than 24 samples per batch decreases the number of disposable filter-tips required per run.
† There are 32 filter-tips/tip rack.
‡ Number of required filter-tips includes filter-tips for 1 inventory scan per RC.
§ There are 28 sample prep cartridges/unit box.
¶ There are twelve 8-Rod Covers/unit box.

Protocol circDNA_8000_DSP

Plasticware	One batch 24 samples*	Two batches 48 samples*
Disposable filter-tips, 200 µL†‡	28	56
Disposable filter-tips, 1500 µL†‡	184	364
Sample prep cartridges§	24	48
8-Rod Covers¶	3	6

* Use of fewer than 24 samples per batch decreases the number of disposable filter-tips required per run.
† There are 32 filter-tips/tip rack.
‡ Number of required filter-tips includes filter-tips for 1 inventory scan per RC.
§ There are 28 sample prep cartridges/unit box.
¶ There are twelve 8-Rod Covers/unit box.

Protocol circDNA_10000_DSP

Plasticware	One batch 24 samples*	Two batches 48 samples*
Disposable filter-tips, 200 µL†‡	28	56
Disposable filter-tips, 1500 µL†‡	224	448
Sample prep cartridges§	27	54
8-Rod Covers¶	3	6

* Use of fewer than 24 samples per batch decreases the number of disposable filter-tips required per run.
† There are 32 filter-tips/tip rack.
‡ Number of required filter-tips includes filter-tips for 1 inventory scan per RC.
§ 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, for example, number of internal controls used per batch. We recommend loading 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.

Preparation of sample material

Note: Specimen stability and performance of the nucleic acid extraction highly depend on various factors, such as specimen collection device and method, storage temperature, freeze-thaw cycles, and transport conditions, and relates to the specific downstream application. It has been established for the QIAsymphony DSP Circulating DNA Kit in conjunction with exemplary specimen collection devices, and downstream applications. It is the responsibility of the user to consult the instructions for use of the specific specimen collection device and downstream application used in their laboratory and/or validate the whole workflow to establish appropriate conditions.

For general collection, transport, and storage recommendations refer to the approved CLSI guideline MM13-A "Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods". Furthermore, the manufacturer's instructions for the selected sample collection device shall be followed during sample preparation, storage, transport, and general handling.

Human plasma

When using blood collection tubes with ccfDNA profile stabilizers, the manufacturer's instructions to perform the plasma preparation, storage, transport, and general handling shall be followed. When using blood collection tubes without ccfDNA profile stabilizers, and if instructions for plasma preparation, storage, transport, and general handling are available from the provider of the dedicated examination

procedure, these shall be followed. For more details refer to ISO 20186-3:2019 (E) Molecular in vitro diagnostic examinations – Specifications for pre-examination processes for venous whole blood – Part 3: Isolated circulating cell free DNA from plasma.

Independent of the blood collection tube manufacturer's instructions following aspects should be considered according to ISO 20186-3:2019 (E) for automated ccfDNA extraction from plasma using the QIAsymphony DSP Circulating DNA Kit and the QIAsymphony SP instrument.

Blood samples without ccfDNA profile stabilizer can be used for plasma preparation. Plasma prepared from tubes with ccfDNA profile stabilizer can also be used.

It is recommended to perform plasma separation immediately after blood donation when using EDTA 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 min at room temperature (15–25°C) after initial plasma generation.

Repeated freezing–thawing leads to denaturation and precipitation of proteins, potentially resulting in reduced yields of circulating cell-free nucleic acids. It is recommended to thaw plasma in a water bath at 30°C for 30 min. If cryoprecipitates are visible in the samples, they must be removed before loading the sample on the instrument. Cryoprecipitates can be resolved by vortexing the sample (ensure that foam, if visible on top of the sample, is removed before loading the sample on the instrument). Alternatively, cryoprecipitates can be removed by centrifugation and transfer of the supernatant without disturbing the pellet to a secondary sample tube (see the labware list that 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 ccfDNA after urine collection, it is strongly recommended to stabilize urine samples immediately. Exemplary downstream applications were utilized for the QIAsymphony DSP Circulating DNA Kits to establish recommendations for urine handling and stabilization. Although the kit is used as a front-end for multiple downstream applications, urine handling needs to be established for any such workflow as part of the downstream application development. Alternatively, when using a commercially available cfDNA profile stabilizer for urine, the manufacturer's instructions shall be followed.

Human urine stabilized

Stabilized urine samples require no sample pretreatment. After stabilization, urine samples shall be centrifuged at low speed (1900 x *g*) for 10-15 min at room temperature (15–25°C) to remove cells prior to extraction of ccfDNA. 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 that 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 (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 10-15 min 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 1500 µL urine (circDNA_1000_DSP), 2500 µL urine (circDNA_2000_DSP), 4500 µl urine (circDNA_4000_DSP), 6500 µL urine (circDNA_6000_DSP), 8500 µL (circDNA_8000_DSP), or 10,500 µL (circDNA_10000_DSP) with 150 µL, 250 µL, 450 µL, 650 µL, 850 µL, or 1050 µL Buffer ATL, respectively.

Incubate the samples at room temperature (15–25°C) for 1 hour.

Centrifuge samples at 1900 x *g* for 10 min 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 that can be found under the resource tab of the product page on **www.qiagen.com**).

Important: Stability and integrity of ccfDNA 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.

Important points before loading the samples

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

Storage of eluates

Note: Eluate stability highly depends on various factors and relates to the specific downstream application. It has been established for the QIAsymphony DSP Circulating DNA Kits in conjunction with exemplary downstream applications. It is the responsibility of the user to consult the instructions for use of the specific downstream application used in their laboratory and/or validate the whole workflow to establish appropriate storage conditions.

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.

Limitations - Interfering substances

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

Symbols

The following symbols appear in the instructions for use or on the packaging and labeling:

Symbol	Symbol definition
CE	This product fulfills the requirements of the European Regulation 2017/746 for in vitro diagnostic medical devices.
IVD	In vitro diagnostic medical device
REF	Catalog number
Rn	R is for revision of the Instructions for Use and n is the revision number
	Manufacturer

Revision history

Revision	Description
R1, June 2022	Version 2, Revision 1 • Update to version 2 for compliance to IVDR • Wording for Specimen handling updated to consider ISO 20186-3:2019 (E) Molecular in vitro diagnostic examinations – Specifications for pre-examination processes for venous whole blood – Part 3: Isolated circulating cell free DNA from plasma
R2, January 2023	Version 2, Revision 2 • Update to add BioScript for 1 mL sample volume (circDNA 1000 DSP) • Update to V3 for circDNA_2000 and circDNA_4000
R3, June 2024	• Document version was removed from revision history • Added the QIAsymphony DSP Circulating DNA Maxi Kit (192) and QIAsymphony DSP Circulating DNA Kit (96) • Update to V2 for circDNA_1000 and update to V4 for circDNA_2000 and circDNA_4000 • Added BioScript for 6 mL, 8 mL, and 10 mL sample volume (circDNA 6000 DSP, circDNA 8000 DSP and circDNA 10000 DSP)

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.

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