June 2022

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

circDNA_2000_DSP_V2 and circDNA_4000_DSP_V2

Version 2

IVD

For In Vitro Diagnostic Use

For use with QIAsymphony DSP Circulating DNA Kit

CE

REF 937556

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R1

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 | |
| **Catalog no.** | 937556 | |
| **Sample material** | Human plasma:<br>• From blood collection tubes with ccfDNA profile stabilizers (e.g., Cell-Free DNA BCT®, Streck®)<br>• from blood collection tubes without ccfDNA profile stabilizers (e.g., EDTA)<br>Human urine:<br>• With cfDNA profile stabilizers<br>• Without cfDNA profile stabilizers | |
| **Protocol name** | circDNA_2000_DSP_V2 | circDNA_4000_DSP_V2 |
| **Default Assay Control Set** | ACS_circDNA_2000_DSP_V2 | ACS_circDNA_4000_DSP_V2 |
| **Elution Volume** | 60 µl | 60 µl |
| **Required software version** | Version 4.0 or higher | Version 5.0 or higher |
| **Required software configuration for IVD use** | 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<br>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** | n/a |
| **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** | n/a |
| **Other** | Proteinase K needs to be added in slot A (position 1, 2, and/or 3) |

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, 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_2000_DSP (µl) | circDNA_4000_DSP (µl) |
|---|---|---|
| 8 | 1980 | 2860 |
| 24 | 3740 | 6380 |
| 48 | 6380 | 11 660 |
| 72 | 9020 | 18 040† |
| 96 | 11 660 | 23 320† |

* 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 (RC) |
| **Position B1** | n/a |
| **Tip rack holder 1–18** | 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** | 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_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†‡ | 56 | 112 | 168 | 224 |
| 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_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†‡ | 96 | 192 | 288 | 384 |
| Sample prep cartridges§ | 18 | 36 | 54 | 72 |
| 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.

**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.

## 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

**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 Kit 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.

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

## Preparation of sample material

**Note**: Sample stability highly depends on various factors and relates to the specific downstream application. It has been established for the QIAsymphony DSP Circulating DNA Kit 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.

### 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 (in example EDTA blood collection tube). Plasma prepared from tubes with ccfDNA profile stabilizer can also be used (in example Cell-Free DNA BCT from Streck).

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 up to 24 months, it is recommended to freeze aliquots at –20°C or –80°C. Frozen plasma must not be thawed more than 3 times.

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 vortex 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 Kit 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 may be stored at room temperature (15–25°C) or at 2–8°C for up to 7 days. For longer storage up to 24 months, 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 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 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 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.

## 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 |
|---|---|
|  <N> | Contains reagents sufficient for <N> reactions |
|  | Use by |
|  | 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 |
| LOT | Lot number |
| MAT | Material number (i.e., component labeling) |
| COMP | Components |
| CONT | Contains |
| NUM | Number |
| GTIN | Global Trade Item Number |
| Rn | R is for revision of the Instructions for Use and n is the revision number |
|  | Temperature limitation |
|  | Manufacturer |
|  | Consult instructions for use |
|  | Warning/caution |
| PROTK | Proteinase K |
| WELL | Well number (i.e., reagent cartridge well) |
| REAG CART | Reagent cartridge |
| Sodium azide | Sodium azide |
| EtOH | Ethanol |
| UDI | Unique device identifier |

## Revision history

| Revision | Description |
|---|---|
| R1, June 2022 | Version 2, Revision 1<br>• Update to version 2 for compliance to IVDR<br>• 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 |

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.