June 2024

QIAsymphony® DSP Circulating DNA Kit Instructions for Use (Handbook)

IVD

For In Vitro Diagnostic Use

	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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Sample to Insight

Contents

Intended Use

The QIAsymphony DSP Circulating DNA Kit utilizes magnetic-particle technology for automated isolation and purification of human circulating cell-free DNA from biological specimens.

The QIAsymphony DSP Circulating DNA Kit is intended for in vitro diagnostic use.

Intended User

The QIAsymphony DSP Circulating DNA Kit is intended to be used by professional users, such as technicians and physicians who are trained in molecular biological techniques.

Description and Principle

QIAsymphony technology combines the speed and efficiency of anion exchange-based nucleic acid purification with the convenient handling of magnetic particles (Figure 1, below). The purification procedure is designed to ensure safe and reproducible handling of potentially infectious samples and comprises 3 steps: bind, wash, and elute (see flowchart on page 6). The user can choose between different sample input volumes.

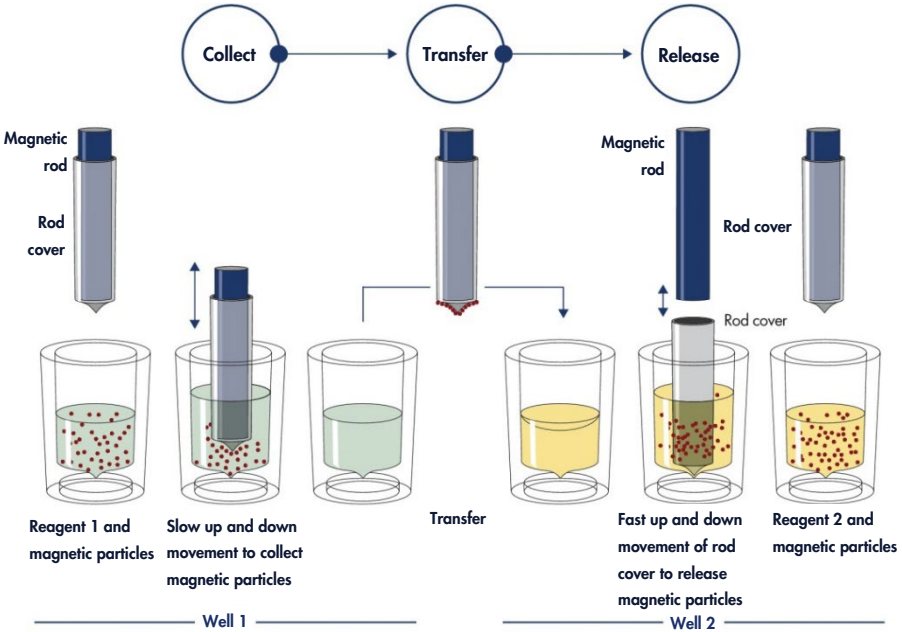
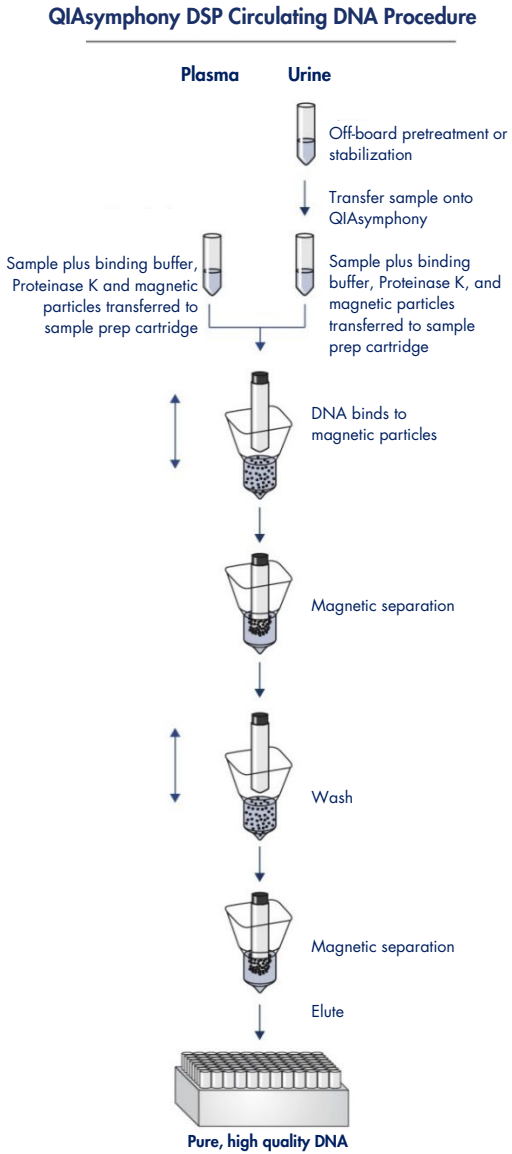


Figure 1. Schematic diagram of the QIAsymphony SP principle. The QIAsymphony SP processes a sample containing magnetic particles as follows: a magnetic rod protected by a rod cover enters a well containing the sample and attracts the magnetic particles. The magnetic rod cover is positioned above another well, and the magnetic particles are released. These steps are repeated several times during sample processing. The QIAsymphony SP uses a magnetic head containing an array of 24 magnetic rods and can therefore process up to 24 samples simultaneously.


QIAsymphony DSP Circulating DNA Procedure
Plasma
Urine
Off-board pretreatment or stabilization
Transfer sample onto QIAsymphony
Sample plus binding buffer, Proteinase K and magnetic particles transferred to sample prep cartridge
Sample plus binding buffer, Proteinase K, and magnetic particles transferred to sample prep cartridge
DNA binds to magnetic particles
Magnetic separation
Wash
Magnetic separation
Elute
Pure, high quality DNA

Summary and explanation

Circulating cell-free nucleic acids (ccfNAs) are present in plasma or urine usually as short fragments, <1000 bp (DNA) and <1000 nt (RNA). The concentration of ccfNAs in biological fluids such as plasma or urine is usually low and varies considerably between individuals. For ccfNA, the concentration can range from 1 to 100 ng/mL. The QIAsymphony DSP Circulating DNA system constitutes a ready-to-use in vitro system for the qualitative purification of human circulating cell-free DNA (ccfDNA) from human plasma and urine using the QIAsymphony SP instrument.

The QIAsymphony DSP Circulating DNA Kit provides reagents for fully automated and simultaneous purification of human ccfDNA from human plasma and urine. A performance characteristic for every blood collection tube has not been established and must be validated by the user. Magnetic-particle technology enables purification of high-quality nucleic acids that are free of proteins, nucleases, and other impurities. The purified ccfDNA is compatible with a wide range of downstream applications. The QIAsymphony SP performs all steps of the purification procedure. Up to 96 samples, in batches of 24, are processed in a single run. Urine samples may require manual sample pretreatment.

Materials Provided

Kit contents

QIAsymphony DSP Circulating DNA Kit	(96)	(192)	Maxi (192)
Catalog no.	937555	937556	937566
Number of reactions	96 (2 mL, 4 mL, 6 mL, 8 mL, and 10 mL sample volume)	192 (2 mL and 4 mL sample volume)	192 (6 mL, 8 mL, and 10 mL sample volume)
	192 (1 mL sample volume)	384 (1 mL sample volume)	

Abbreviations	Identity	Quantity		
RC REAG CART	Reagent cartridge*	2	2	2
PROTK PROTK	QIAGEN Proteinase K	3 x 10 mL†	6 x 10 mL	13 x 10 mL
PL	Piercing lid	2	2	2
RSS	Reuse Seal Set‡	2	2	2
	Instructions for Use (Handbook)	1	1	1

* Contains sodium azide as a preservative.

† Additional Proteinase K bottles have to be ordered for 6 mL, 8 mL, and 10 mL sample volume to process 96 samples in total (refer to point additional reagents).

‡ A Reuse Seal Set contains 8 Reuse Seal Strips.

Components of the kit

The principal components of the kit containing active ingredients are explained below.

Reagent	Components	Concentration (w/w) [%]*
RC (Reagent cartridge)	Nonionic detergent	≥0.5 to <10 [w/w]
	Anion exchange magnetic particle	n/a
	NaOH	≥0.05 to <0.1 [w/w]
	Ethanol	≥70 to <90 [v/v]
QIAGEN Proteinase K	Proteinase K	≥1 to <3 % [w/w]

* Maximum concentration in a single well.

Controls and calibrators

To minimize the risk of a negative impact on the diagnostic results, adequate controls for downstream applications should be used.

Materials Required but Not Provided

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs) available from the product supplier.

Additional reagents

- Buffer ATL (for pretreatment of urine samples; cat. no. 939016)
- Proteinase K (cat. no 19134) for 6–10 mL sample volume for use with the QIAsymphony DSP Circulating DNA Kit (96)
- Phosphate-buffered saline (PBS, may be required for topping up sample volumes)

For additional information how much Proteinase K muss be ordered, please refer to the protocol sheet, which can be found under the resource tab of the product page on **www.qiagen.com**

For additional information needed for pretreatment and stabilization of urine samples, please refer to the protocol sheet, which can be found under the Resources tab of the product page on **www.qiagen.com**

Consumables

- Sample Prep Cartridges, 8-well cartridges (cat. no. 997002)
- 8-Rod Covers (cat. no. 997004)
- Filter-Tips, 200 µL (cat. no. 990332) and 1500 µL (cat. no. 997024)
- Sample tubes. For compatible primary and secondary tube formats, see the labware list, which can be found under the Resources tab of the product page on **www.qiagen.com**
- Elution tubes or plates. For compatible elution tube and plate formats, see the labware list, which can be found under the Resources tab of the product page on **www.qiagen.com**

- Pipette tips for adjustable pipettes (to prevent cross-contamination, we strongly recommend the use of pipette tips with aerosol barriers)

Equipment

Prior to use, ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

- QIAsymphony SP (cat. no. 9001297)
- Vortexer
- Pipettes (adjustable)

Protocol and labware

Next to the handbook, the instructions for use consist of the protocol sheet, labware list, and performance characteristics, which can be found under the Resources tab of the product page on **www.qiagen.com**

Warnings and Precautions

Please be aware that you may be required to consult your local regulations for reporting serious incidents that have occurred in relation to the device to the manufacturer and/or its authorized representative and the regulatory authority in which the user and/or the patient is established.

For in vitro diagnostic use

Read all instructions carefully before using the kit.

Please be aware of following remaining risks:

- Sample IDs can also be entered manually (for details, refer to the *QIAsymphony SP User Manual*). If wrong ID data are entered manually, wrong correlation between sample and patient can occur.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety**, where you can find, view, and print the SDS for each QIAGEN kit and kit component.

- All chemicals and biological materials are potentially hazardous. Samples are potentially infectious and must be handled and discarded according to your local safety procedures.
- QIAGEN has not tested the liquid waste generated by the QIAsymphony DSP Circulating DNA Kit procedure for residual infectious materials. Therefore, universal precautions (gloves, lab coats, and eye protection) for handling potentially infectious human source

material should be employed while working with this product, and liquid waste must be considered infectious and be handled and discarded according to local safety regulations.

- Buffers in the reagent cartridge contain sodium azide. If buffers of the kit are spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

WARNING 	**Risk of personal injury** Do not add bleach or acidic solutions directly to the sample preparation waste.

Emergency information

CHEMTREC
USA & Canada 1-800-424-9300
Outside USA & Canada +1 703-527-3887

Precautions

The following hazard and precautionary statements apply to components of QIAsymphony DSP Circulating DNA Kit.

MBS3

Sodium azide

Contains: Sodium azide. May be harmful if swallowed. Call a POISON CENTER or doctor/physician if you feel unwell.

Proteinase K

Contains: Proteinase K. Danger! Causes mild skin irritation. May cause allergy or asthma symptoms or breathing difficulties if inhaled. Avoid breathing dust/fume/gas/mis/vapors/spray. Dispose of contents/container to an approved waste disposal plant. If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician. IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing. Wear respiratory protection.

QSW9

Contains: ethanol. Danger! Causes serious eye irritation. Highly flammable liquid and vapor. Dispose of contents/container to an approved waste disposal plant. If eye irritation persists: Get medical advice/attention. Keep away from heat/sparks/open flames/hot surfaces. - No smoking. Store in a well-ventilated place. Keep cool. Wear protective gloves/protective clothing/eye protection/face protection.

Disposal

The waste contains samples and reagents. This waste may contain toxic or infectious material and must be disposed properly. Refer to your local safety regulations for proper disposal procedures.

For more information, please consult the appropriate safety data sheets (SDSs). These are available online in PDF format at **www.qiagen.com/safety** where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Reagent Storage and Handling

Attention should be paid to expiration dates and storage conditions printed on the box. Do not use expired or incorrectly stored components.

The QIAsymphony DSP Circulating DNA Kits should be stored upright at room temperature (15–25°C). Storage at temperatures below 15°C can lead to formation of precipitates in buffers (see Important Points before Starting on page 23).

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

When stored properly, the kit is stable until the expiration date on the kit box.

Note: The label on the QIAsymphony DSP Circulating DNA Kit box displays the expiration date of the kit. The result file documents the expiration dates for only the reagent cartridge.

In-use stability

Partially used reagent cartridges can be stored for a maximum of 4 weeks, upright at room temperature (15–25°C), enabling cost-efficient reuse of reagents and more flexible sample processing. If a reagent cartridge is partially used, replace the cover of the trough containing the magnetic particles and seal the reagent cartridge with the provided Reuse Seal Strips (RSS) immediately after the end of the protocol run to avoid evaporation.

To avoid reagent evaporation, the reagent cartridge should be open for a maximum of 15 hours (including run times) at a maximum environmental temperature of 32°C. Incorrect storage of the kit components can lead to accelerated aging of buffers.

Running batches with low sample numbers (<24) will increase both the time that the reagent cartridge (RC) is open and the required buffer volumes, potentially reducing the total number of sample preparations possible per cartridge.

Avoid exposure of the reagent cartridges to UV light (e.g., used for decontamination) as exposure may cause accelerated aging of the reagent cartridges and buffers.

Specimen Collection, Storage, and Handling

Note: Specimen stability and performance of the nucleic acid extraction highly depends 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 Kits 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 more information about the automated procedure (including information about sample tubes that can be used with specific protocols), specimen storage, handling, and specific sample pretreatments, see the relevant protocol sheet and labware list, which can be found under the Resources tab of the product page on **www.qiagen.com**

Procedure

Automated purification on QIAsymphony SP

The QIAsymphony SP makes automated sample preparation easy and convenient. Samples, reagents and consumables, and eluates are separated in different drawers. Simply load samples, reagents provided in special cartridges, and preracked consumables in the appropriate drawer before a run. Start the protocol and remove purified DNA from the "Eluate" drawer after processing. Refer to the user manuals supplied with your instrument for operating instructions.

Note: Optional maintenance is not mandatory for instrument function but is highly recommended to reduce risk of contamination.

The range of protocols available is continually expanding, and additional QIAGEN protocols can be downloaded free of charge at **www.qiagen.com** under the Resources tab of the individual kits.

Loading reagent cartridges into the "Reagents and Consumables" drawer

Reagents for purification of DNA are contained in a reagent cartridge (Figure 2, page 19). Each trough of the reagent cartridge contains a particular reagent, such as magnetic particles, binding buffer, wash buffer, or elution buffer. Partially used reagent cartridges can be reclosed with Reuse Seal Strips to avoid evaporation generation can be stored until needed again, see "Reagent Storage and Handling", page 16.

Figure 2. QIAsymphony reagent cartridge. The reagent cartridge contains all reagents required for the protocol run.

Before starting the procedure, place the reagent cartridge into the reagent cartridge holder. Before using a reagent cartridge for the first time, place the piercing lid (PL) on top of the reagent cartridge (Figure 2).

> **Note:** The piercing lid is sharp. Take care when placing it onto the reagent cartridge. Make sure to place the piercing lid onto the reagent cartridge in the correct orientation and gently push it downward until it clicks into place. The RC is pierced by the QIAsymphony SP instrument.

Before use, remove the magnetic-particle trough from the reagent cartridge frame, vortex it vigorously for at least 3 min to ensure that the magnetic particles are fully resuspended, then replace it in the reagent cartridge frame.

> **Note**: Magnetic particles can change color. This has no influence on performance.

If using partially used RCs, make sure to remove the Reuse Seal Strips.

Remove the magnetic-particle trough foil or cover and subsequently load the reagent cartridge into the "Reagents and Consumables" drawer.

Note: Proteinase K must be added according to information given in the protocol sheet under the Resources tab of the product page on **www.qiagen.com**

Loading plasticware into the "Reagents and Consumables" drawer

Sample prep cartridges, 8-Rod Covers (both preracked in unit boxes), and disposable filter-tips (200 µL tips provided in blue racks and 1500 µL tips provided in black racks) are loaded into the "Reagents and Consumables" drawer.

Note: Make sure that the covers of the unit boxes are removed before loading the unit boxes into the "Reagents and Consumables" drawer.

Note: Tips have filters to help prevent cross-contamination.

Tip rack slots on the QIAsymphony SP worktable can be filled with either type of tip rack. The QIAsymphony SP will identify the type of tips loaded during the inventory scan.

Note: Do not refill tip racks or unit boxes for sample prep cartridges or 8-Rod Covers before starting another protocol run. The QIAsymphony SP can use partially used tip racks and unit boxes.

For the consumables required, see the relevant protocol sheet, which can be found under the Resources tab of the product page on **www.qiagen.com**. For plasticware ordering information, see page 37.

Loading the "Waste" drawer

Sample prep cartridges and 8-Rod Covers used during a run are re-racked in empty unit boxes in the "Waste" drawer. Make sure that the "Waste" drawer contains sufficient empty unit boxes for plastic waste generated during the protocol run.

> **Note**: Make sure that the covers of the unit boxes are removed before loading the unit boxes into the "Waste" drawer. If you are using 8-Rod Cover boxes for collecting used sample prep cartridges and 8-Rod Covers, ensure that the box spacer has been removed.

A bag for used filter-tips must be attached to the front side of the "Waste" drawer.

> **Note**: The presence of a tip disposal bag is not checked by the system. Make sure that the tip disposal bag is properly attached before starting a protocol run. For more information, see the user manuals provided with your instrument. Empty the tip bag after a maximum of 96 samples have been processed to avoid a tip jam.

A waste container collects liquid waste generated during the purification procedure. The "Waste" drawer can only be closed if the waste container is in place. Dispose of the liquid waste according to your local safety and environment regulations. Do not autoclave the filled waste bottle. Empty the waste bottle at the latest after a maximum of 96 samples have been processed.

Loading the "Eluate" drawer

Load the required elution rack into the "Eluate" drawer. As long-term storage of eluates in the "Eluate" drawer may lead to evaporation or condensation, the cooling position must be used. Only use "Elution slot 1" with the corresponding cooling adapter.

Inventory scan

Before starting a run, the instrument checks that sufficient consumables for the queued batch(es) have been loaded into the corresponding drawers.

Preparation of sample material

The QIAsymphony DSP Circulating DNA Kits are designed for automated purification of human circulating cell-free DNA from human plasma and urine.

Prevent formation of foam in or on the samples. Foam on samples can lead to pipetting of wrong sample volume. Depending on the starting material, sample pretreatment may be required. Samples should be equilibrated to room temperature (15–25°C) before starting the run.

For more information about the automated procedure (including information about sample tubes that can be used with specific protocols) and specific sample pretreatments, see the relevant protocol sheet and labware list, which can be found under the Resources tab of the product page on **www.qiagen.com**.

Storing DNA

> **Note**: Eluate stability highly depends on various factors and relates to the specific downstream application. It has been established for the QS 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.

Storage conditions and duration of the purified nucleic acid depend on the specimen type used.

Protocol: Purification of circulating cell-free DNA

Protocol overview

Table 1. Protocol overview

Sample	Sample volume (µL)	Elution volume (µL)	QIAsymphony SP protocol
Plasma, urine	1000	60	circDNA_1000_DSP
Plasma, urine	2000	60	circDNA_2000_DSP
Plasma, urine	4000	60	circDNA_4000_DSP
Plasma, urine	6000	60	circDNA_6000_DSP
Plasma, urine	8000	60	circDNA_8000_DSP
Plasma, urine	10000	60	circDNA_10000_DSP

Detailed information are given in the protocol sheets and labware list, which can be found under the Resources tab of the product page on **www.qiagen.com**.

The following is a general protocol for using the QIAsymphony DSP Kits. Detailed information for each protocol, including volumes and tubes, is provided in the protocol sheets and labware list, which can be found under the Resources tab of the product page on **www.qiagen.com**.

Important points before starting

- After receiving the kit, check the kit components for damage. Do not use damaged kit components, since their use may lead to poor kit performance, user injury, or damage to the instrument.
- Make sure that you are familiar with operating the QIAsymphony SP. Refer to the user manuals supplied with your instrument for operating instructions.
- Optional maintenance is not mandatory for instrument function but is highly recommended to reduce risk of contamination.
- Before beginning the procedure, read “Description and Principle”, starting on page 5.

- Make sure that you are familiar with the protocol sheet corresponding to the procedure you want to use. Protocol sheets can be found under the Resources tab of the product page on **www.qiagen.com**.
- Avoid vigorous shaking of the reagent cartridge; otherwise, foam may be generated, which can lead to liquid-level detection problems.
- Quality control procedures at QIAGEN employ functional kit release testing for each individual kit lot. Therefore, do not mix reagents from different kit lots, and do not combine individual reagents from different reagent lots.
- Before starting a pretreatment that requires Buffer ATL, check whether precipitate has formed in Buffer ATL. If necessary, dissolve precipitate by heating at 70°C with gentle agitation in a water bath.* Aspirate bubbles from the surface of Buffer ATL.

Things to do before starting

- Before starting the procedure, make sure that the magnetic particles are fully resuspended. Vortex the trough containing the magnetic particles vigorously for at least 3 min before use.
- Make sure that the piercing lid is placed on the reagent cartridge and the lid of the magnetic-particle trough has been removed or, if using a partially used reagent cartridge, make sure the Reuse Seal Strips have been removed.
- Proteinase K is not included in the reagent cartridge but has to be provided by the user (sample drawer, slot A, position 1, 2, and/or 3). Make sure that correct Proteinase K volume is available. (For detailed information, see the protocol sheet which can be found under the Resources tab of the product page on **www.qiagen.com**).
- If samples are bar coded, orient samples in the tube carrier so that the bar codes face the bar code reader at the left side of the QIAsymphony SP.

* Ensure that instruments have been checked, maintained, and calibrated regularly according to the manufacturer's instructions.

- For information about sample tubes compatible with a certain protocol, see the corresponding labware list, which can be found under the Resources tab of the product page on **www.qiagen.com**.
- For information about minimum sample volumes for secondary tubes, see the corresponding labware list, which can be found under the Resources tab of the product page on **www.qiagen.com**.

Procedure

1. Close all drawers and the hood.
2. Power ON the QIAsymphony SP and wait until the **Sample Preparation** screen appears and the initialization procedure has finished.

   The power switch is located at the bottom, left corner of the QIAsymphony SP.
3. Log on to the instrument.
4. Load the required elution rack into the "Eluate" drawer.

   Do not load a 96-well plate onto "Elution slot 4". "Elution slot 1", with the corresponding cooling adapter, must be used.

   When using a 96-well plate, make sure that the plate is in the correct orientation, as incorrect placement may cause sample mix-up in downstream analysis.

   When using the Elution Microtubes CL rack, remove the bottom by twisting the rack until the bottom comes off.
5. Make sure the "Waste" drawer is properly prepared and perform an inventory scan of the "Waste" drawer, including the tip chute and liquid waste. Replace the tip disposal bag if necessary.
6. Load the required reagent cartridge(s) and consumables into the "Reagents and Consumables" drawer.
7. Perform an inventory scan of the "Reagents and Consumables" drawer.
8. Place the samples into the appropriate sample carrier and load them into the "Sample" drawer.

Note: To ensure correct liquid level detection, push the tubes down to the bottom of the tube carrier or insert, if inserts are used.

9. Using the touchscreen, enter the required information for each batch of samples and for Proteinase K to be processed.

   Enter the following information:

   - Sample information (depending on sample racks used)
   - Protocol to be run (Assay Control Set)
   - Elution volume and output position

   After information about the batch has been entered, the status changes from LOADED to QUEUED. As soon as one batch is queued, the Run button appears.

10. Place the Proteinase K into the appropriate sample carrier on position 1, 2, and/or 3, and load them into slot A of the "Sample" drawer.
11. Define the Proteinase K by pressing the **IC** button.
12. Press the **Run** button to start the purification procedure.

    All processing steps are fully automated. At the end of the protocol run, the status of the batch changes from RUNNING to COMPLETED.

13. Retrieve the elution rack containing the purified nucleic acids from the "Eluate" drawer.
14. The DNA is ready to use or can be stored.

    We recommend removing the eluate plate from the "Eluate" drawer immediately after the run has finished. Depending on temperature and humidity, elution plates left in the QIAsymphony SP after the run is completed may exhibit condensation or evaporation.

    In general, magnetic particles are not carried over into eluates. If carryover does occur, magnetic particles in eluates will not affect most downstream applications.

    If magnetic particles need to be removed before performing downstream applications, tubes or plates containing eluates should first be placed in a suitable magnet and the eluates transferred to a clean tube (see "Troubleshooting Guide", page 30).

Result files are generated for each elution plate.

15. If a reagent cartridge is only partially used, seal it with the provided Reuse Seal Strips after the end of the protocol run to avoid evaporation.

    Note: For more information about storage of partially used reagent cartridges, see "Reagent Storage and Handling", page 16.

16. Discard used sample tubes and waste according to your local safety regulations.

    See "Warnings and Precautions", page 12, for safety information.

17. Clean the QIAsymphony SP.

    Follow the maintenance instructions in the user manuals supplied with your instrument. Make sure to clean the tip guards regularly to minimize the risk of cross-contamination.

18. Close the instrument drawers and power OFF the QIAsymphony SP.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIAsymphony DSP Circulating DNA Kit is tested against predetermined specifications to ensure consistent product quality.

Limitations

System performance has been established in performance evaluation studies purifying human ccfDNA from human plasma and urine. Blood has been collected in blood collection tubes without ccfDNA profile stabilizers (EDTA tubes), and blood collection tubes with ccfDNA profile stabilizers (PAXgene® Blood ccfDNA Tube, PreAnalytiX; Cell-Free DNA BCT®, Streck®).

It is the user's responsibility to validate system performance for any procedures used in their laboratory that are not covered by the QIAGEN performance evaluation studies.

To minimize the risk of a negative impact on the diagnostic results, adequate controls for downstream applications should be used. For further validation, the guidelines of the International Conference on Harmonisation of Technical Requirements (ICH) in *ICH Q2 (R1) Validation of Analytical Procedures: Text and Methodology* are recommended.

Any diagnostic results that are generated must be interpreted in conjunction with other clinical or laboratory findings.

For more information about Limitations, see the relevant protocol sheet, which can be found under the Resources tab of the product page on **www.qiagen.com**.

Performance Characteristics

The performance characteristics can be found under the Resources tab of the product page on **www.qiagen.com**.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

		Comments and suggestions
General handling		
Error message displayed on the touchscreen		If an error message is displayed during a protocol, refer to the user manuals supplied with your instrument.
Precipitate in reagent trough of opened cartridge of the QIAsymphony DSP kit		
a)	Buffer evaporation	Excessive evaporation may lead to increased salt concentration in buffers. Discard the reagent cartridge. Make sure to seal buffer troughs of a partially used reagent cartridge with Reuse Seal Strips when not being used for purification.
b)	Storage of reagent cartridge	Storage of reagent cartridge below 15°C may lead to formation of precipitates.
Low yield of DNA		
a)	Magnetic particles were not completely resuspended	Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex for at least 3 min before use.
b)	Clogging of pipette tip due to insoluble material	Insoluble material was not removed from the sample prior to starting the QIAsymphony purification procedure. If required, use pretreatment procedures as described in the corresponding protocol sheet, which can be found under the Resources tab of the product page on **www.qiagen.com**.
c)	Sample material contains low concentration of ccfDNA	Due to very low amounts of ccfDNA in sample material, it is possible not to detect DNA concentration depending on the quantification method used. Use of sensitive qPCR is recommended to check DNA concentration in eluates.
d)	Incomplete reclosing of reagent cartridge	Exchange with surrounding air may lead to reduced stability of buffers leading to reduced efficiency of ccfDNA extraction with a partially used reagent cartridge. Make sure to carefully seal buffer troughs of a partially used reagent cartridge with Reuse Seal Strips when not being used for purification.

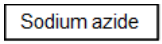
		Comments and suggestions
e)	Rapid degradation of ccfDNA in non-stabilized urine sample	Due to rapid degradation of ccfDNA in non-stabilized urine samples after sample collection, it is possible to detect no/low DNA concentration in eluates. It is recommended to stabilize urine sample as described in the corresponding protocol sheet. Alternatively, subject urine samples immediately after collection and centrifugation to ATL-pretreatment and subsequent DNA extraction on the instrument as described in the corresponding protocol sheet.
No/incomplete sample transfer		
a)	Incorrect sample volume is loaded	**circDNA_1000_DSP**: If less than 1.2 mL (Sarstedt tube) and 1.4 mL (BD tube) sample volume is loaded, there is an increased risk of reporting error code 1400043 (enable less sample) off the sample. If less than 0.7 mL (Sarstedt tube) and 0.9 mL (BD tube) sample volume is loaded, there is an increased risk of invalid flagging and no transfer of the sample. **circDNA_2000_DSP**: If less than 2.4 mL sample volume is loaded, there is an increased risk of reporting error code 140043 (enable less sample) off the sample. If less than 1.4 mL sample volume is loaded, there is an increased risk of invalid flagging and no transfer of the sample. **circDNA_4000_DSP**: If less than 4.5 mL sample volume is loaded, there is an increased risk of reporting error code 140043 (enable less sample) off the sample. If less than 3.6 mL sample volume is loaded, there is an increased risk of invalid flagging and no transfer of the sample. **circDNA_6000_DSP**: If less than 6.6 mL sample volume is loaded, there is an increased risk of reporting error code 140043 (enable less sample) off the sample. If less than 5.9 mL sample volume is loaded, there is an increased risk of invalid flagging and no transfer of the sample. **circDNA_8000_DSP**: If less than 8.6 mL sample volume is loaded, there is an increased risk of reporting error code 140043 (enable less sample) off the sample. If less than 7.8 mL sample volume is loaded, there is an increased risk of invalid flagging and no transfer of the sample. **circDNA_10000_DSP**: If less than 10.8 mL sample volume is loaded, there is an increased risk of reporting error code 140043 (enable less sample) off the sample. If less than 9.9 mL sample volume is loaded, there is an increased risk of invalid flagging and no transfer of the sample. Load the correct sample volume as described in the corresponding labware list. If insufficient sample is available, add PBS to the sample up to the required sample volume before loading the sample.
b)	Bubbles and/or foam in sample tube	Bubbles or foam in the sample and/or sample input tube may result in false liquid level detection and subsequent incomplete sample transfer. Remove bubbles from the sample tube.

	Comments and suggestions
Brown pellet in eluate visible	
Bead carryover into the eluate	If bead carryover does occur, magnetic particles in eluates will not affect most downstream applications. If magnetic particles need to be removed, apply the tube containing the DNA to a suitable magnetic separator until the magnetic particles are separated. If a suitable magnetic separator is not available, centrifuge the tube containing the DNA for 1 minute at full speed in a microcentrifuge to pellet any remaining magnetic particles.

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.
	In vitro diagnostic medical device
	Catalog number
	Lot number
	Material number (i.e., component labeling)
	Components
	Contains
	Number
	Global Trade Item Number
Rn	R is for revision of the Instructions for Use and n is the revision number

Symbol	Symbol definition
	Temperature limitation
	Manufacturer
	Consult instructions for use
	Warning/caution
	Well number (i.e., reagent cartridge well)
	Sodium azide
	Ethanol
	Unique device identifier
	Sharp edge
	Volume
	This way up

Contact Information

For technical assistance and more information, please see our Technical Support Center at **www.qiagen.com/Support**, call 00800-22-44-6000, or contact one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit **www.qiagen.com**).

Appendix: Quantification of Circulating Cell-Free DNA

Because of very low concentrations of ccfDNA in sample materials, measurement of DNA with a spectrophotometer is not recommended. For determination of concentration of circulating cell-free DNA, a sensitive and accurate fluorescence-based quantitation assay or a PCR assay should be used.

Ordering Information

Product	Contents	Cat. no.
QIAsymphony DSP Circulating DNA Kit	Includes 2 reagent cartridges and Proteinase K Tubes and accessories	937556
QIAsymphony DSP Circulating DNA Maxi Kit (192)	Includes 2 reagent cartridges and Proteinase K Tubes and accessories	937566
QIAsymphony DSP Circulating DNA Kit (96)	Includes 2 reagent cartridges and Proteinase K Tubes and accessories	937555
Related instrument		
QIAsymphony SP	QIAsymphony sample prep module	9001297
Related products		
Buffer ATL (4 x 50 mL)	4 x 50 mL Buffer ATL for pretreatment of urine samples	939016
Proteinase K (10 mL)	1 x 10 mL bottle	19134
Reagent Cartridge Holder (2)	Reagent cartridge holder for use with the QIAsymphony SP	997008
Cooling Adapter, 2 mL, v2, Qsym	Cooling adapter for 2 mL screw-cap tubes. For use in the QIAsymphony "Eluate" drawer	9020674
Cooling Adapter, EMT, v2, Qsym	Cooling adapter for EMT racks. For use in the QIAsymphony SP/AS instruments (software version 3.1 or higher)	9020730

Cooling Adapter, Snap-Cap Microtube QIAsymphony, Qsym	Cooling adapter for 1.5 mL Eppendorf® LoBind Snap Cap Safe-Lock tubes. For use in the QIAsymphony "Eluate" drawer	9020731
Sample Prep Cartridges, 8-well (336)	8-well sample prep cartridges for use with the QIAsymphony SP	997002
8-Rod Covers (144)	8-Rod Covers for use with the QIAsymphony SP	997004
Filter-Tips, 200 µL (1024)	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAcube® and the QIAsymphony SP/AS	990332
Filter-Tips, 1500 µL, Qsym SP (1024)	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAsymphony SP/AS	997024
Tip Disposal Bags (15)	Tip disposal bags for use with the QIAsymphony SP/AS instruments	9013395
Reuse Seal Set (20)	Reuse Seal Set for sealing QIAsymphony reagent cartridges	997006
Elution Microtubes CL (24 x 96)	Non-sterile polypropylene tubes (0.85 mL maximum capacity, less than 0.7 mL storage capacity, 0.4 mL elution capacity); 2304 in racks of 96; includes cap strips	19588

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

Document Revision History

Revision	Description
R1, June 2022	Version 2, Revision 1 • Update to version 2 for compliance to IVDR • Update of Materials Provided (add active ingredients) • Update of Warnings and Precautions • Update of Reagent Storage and Handling • Add section Disposal Update of Troubleshooting guide (added bead carryover)
R2, January 2023	Version 2, Revision 2 • Update to add BioScript for 1 mL sample volume (circDNA_1000_DSP) • Update of Troubleshooting Guide
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) • Added BioScript for 6 mL, 8 mL, and 10 mL sample volume (circDNA 6000 DSP, circDNA 8000 DSP and circDNA 10000 DSP)

Limited License Agreement for QIAsymphony DSP Circulating DNA Kit

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