June 2022

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

DNA_Buffy_Coat_400_V6 DSP protocol

Version 2

IVD

For In Vitro Diagnostic Use

For use with QIAsymphony DSP DNA Midi Kit (96)

CE

REF 937255

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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

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

This protocol is for purification of total genomic and mitochondrial DNA from fresh or frozen human whole blood using the QIAsymphony SP and the QIAsymphony DSP DNA Midi Kit.

Kit	QIAsymphony DSP DNA Midi Kit (cat. no. 937255)
Sample material	Buffy coat (EDTA, citrate, or heparin anti-coagulated)
Protocol name	DNA_BC_400_V6_DSP
Default Assay Control Set	ACS_BC_400_V6_DSP
Editable	Elution volume: 200 and 400 µl
Required software version	Version 4.0 or higher
Required software configuration for IVD use	Default Profile 1

"Sample" drawer

Sample type	Human whole blood (EDTA, citrate, or heparin anti-coagulated)
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	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**.

n/a = not applicable.

"Reagents and Consumables" drawer

Position A1 and/or A2	Reagent cartridge (RC)
Position B1	n/a
Tip rack holder 1–17	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	Empty 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

Plasticware	One batch 24 samples*	Two batches 48 samples*	Three batches 72 samples*	Four batches 96 samples*
Disposable filter-tips, 200 µl†‡	4	4	4	8
Disposable filter-tips, 1500 µl†‡	110	212	314	424
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. We recommend loading the maximum possible number of tips.

Elution volume

The elution volume is selected in the touchscreen. Depending on the sample type and DNA content, the final eluate volume may vary by up to 15 µl less than the selected volume. Because the eluate volume may vary, we recommend checking the actual eluate volume when using an automated assay setup system that does not verify the eluate volume prior to transfer. Elution in lower volumes increases the final DNA concentration but slightly reduces the yield. We recommend using an elution volume appropriate for the intended downstream application.

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.

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.

Buffy coat

Buffy coat is a leukocyte-enriched fraction of whole blood. The efficiency of leukocyte enrichment depends on the procedure used to prepare buffy coat and on the accuracy with which the buffy coat layer is extracted. Prepare buffy coat by centrifuging whole blood samples containing a standard anticoagulant (EDTA, citrate, or heparin) at 900–1100 x *g* for 10 minutes at room temperature (15–25°C). After centrifugation, 3 different fractions are distinguishable: the upper clear layer is plasma; the intermediate layer is buffy coat, containing concentrated leukocytes; and the bottom layer contains concentrated erythrocytes. Approximately 1 ml leukocyte-containing fraction should

be harvested from 10 ml centrifuged whole blood, which, on average, gives a 5–6 times enrichment. For example, 10 ml whole blood with a white blood cell count of 6 x 10^6 cells/ml results in 1 ml buffy coat. Assuming a 5 times enrichment of white blood cells, this results in 3 x 10^7 cells/ml. Therefore, in a protocol that uses 400 µl buffy coat, 1.2 x 10^7 cells will be used.

To avoid overloading the DNA purification procedure, do not prepare buffy coat samples of >10 times enrichment. If buffy coat samples are of >10 times enrichment, dilute the samples to 10 times enrichment or less with PBS or use less starting material in the DNA purification procedure.

Buffy coat samples may be used immediately or stored at –20°C or –80°C for purification of DNA at a later date. Frozen samples should be thawed quickly in a 37°C water bath with mild agitation to ensure thorough mixing and then equilibrated to room temperature (15–25°C) before beginning the procedure. To ensure reliable sample transfer, avoid generating foam in sample tubes. Try to avoid blood clots in the samples and, if necessary, transfer the sample without clots to a fresh tube.

Note: Sample stability highly depends on various factors and relates to the specific downstream application. 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 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 12 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.

For short-term storage eluates might be stored at room temperature for up to 2 weeks. For long-term storage, we recommend storage at 2–8°C, –20°C, or –80°C. Frozen eluates must not be thawed more than three times.

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

Important point before starting

- QIAsymphony magnetic particles may copurify RNA if it is present in the sample. To minimize RNA content in the sample, add RNase A to the sample before starting the procedure. The final RNase A concentration should be 2 mg/ml.

Limitations and interfering substances

Blood samples with high concentrations of triglycerides (>30 g/l) may lead to reduced gDNA yield.

Note: Testing was done using exemplary downstream applications for an assessment of the quality of the extracted nucleic acids. However, different downstream applications may have different requirements with respect to purity (i.e., absence of potential interfering substances), so the identification and testing of relevant substances also needs to be established as part of the downstream application development for any workflow involving the QIAsymphony DSP DNA Midi Kit.

Note: Please note that during development of the QIAsymphony DSP DNA Midi Kit no indications were observed that heparin has an negative impact on the performance. However, ISO 20186-2:2019(E) states that heparin from blood collection tubes may impact the purity of the isolated nucleic acids and possible carryover into eluates could cause inhibitions in some downstream applications. Therefore, it is the user's responsibility to validate if heparin has an negative influence on their workflow.

Symbols

The following symbols appear in this document. For a full list of symbols used in the instructions for use or on the packaging and labeling, please refer to the handbook.

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 IVD • Addition of Limitations and Interfering Substances section • Addition of Storage of eluates section • Addition of Symbols section • Update of Preparation of sample material section

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.