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QIASymphony® DSP Virus/Pathogen Kit Instructions for Use (Protocol Sheet)

Complex800_OBL_V4_DSP protocol

Version 2

IVD

For In Vitro Diagnostic Use

For use with QIASymphony DSP Virus/Pathogen Midi Kit



REF

937055



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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.

General information

The QIAAsymphony DSP Virus/Pathogen Kit is intended for in vitro diagnostic use.

Kit	QIAAsymphony DSP Virus/Pathogen Midi Kit
Sample material	Respiratory and urogenital samples
Protocol name	Complex800_OBL_V4_DSP
Default Assay Control Set	ACS_Complex800_OBL_V4_DSP
Editable	Eluate volume: 60, 85, and 110 µl
Required software version	Version 4.0 or higher
Required software configuration for IVD use	Default Profile 1

“Sample” drawer

Sample type	Urine, urogenital swabs (in transport media, e.g., PreservCyt [®] , UTM, eNAT [™]), and respiratory swabs (dried swabs or in transport media, e.g., UTM, eNAT)
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
Processed sample volume	See the labware list which can be found under the resource tab of the product page on www.qiagen.com for more information
Primary sample tubes	See the labware list which can be found under the resource tab of the product page on www.qiagen.com for more information
Secondary sample tubes	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
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
Other	Carrier RNA–Buffer AVE mix required; use of internal control is optional

“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 µl
Tip rack holder 1–17	Disposable filter-tips, 1500 µl
Unit box holder 1–4	Unit boxes containing sample prep cartridges
Unit box holder 1–4	Unit boxes containing 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

Plasticware	One batch 24 samples*	Two batches 48 samples*	Three batches 72 samples*	Four batches 96 samples*
Disposable filter-tips, 200 µl†‡	96	96	128	128
Disposable filter-tips, 1500 µl†‡	128	192	224	288
Sample prep cartridges§	18	36	54	72
8-Rod Covers¶	3	6	9	12

* Performing more than one inventory scan requires additional disposable filter-tips. Use of less than 24 samples per batch decreases the number of disposable 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 on the touchscreen depending on settings. We recommend loading the maximum possible number of tips.

Selected elution volume

Selected elution volume (µl)*	Initial elution volume (µl)†
60	90
85	115
110	140

* The elution volume selected on the touchscreen. This is the minimum accessible volume of eluate in the final elution tube.

† The initial volume of elution solution required to ensure that the actual volume of eluate is the same as the selected volume.

Preparation of internal control–carrier RNA (CARRIER)–Buffer AVE (AVE) mixture

Selected elution volume (µl)	Volume stock carrier RNA (CARRIER) (µl)	Volume internal control (µl)*	Volume Buffer AVE (AVE) (µl)	Final volume per sample (µl)
60	3	9	108	120
85	3	11.5	105.5	120
110	3	14	103	120

* The calculation of the amount of internal control is based on the initial elution volumes. Additional void volume depends on the type of sample tube used; see the labware list, which can be found under the resource tab of the product page on www.qiagen.com for more information.

Note: The values displayed in the table are for preparation of internal control–carrier RNA (CARRIER) mixture for a downstream assay that requires 0.1 µl internal control/µl eluate.

Off-board lysis

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.

The QIASymphony Complex protocols consist of 4 steps: lyse, bind, wash, and elute. For some samples, it is useful to perform lysis manually, for example, for inactivation of pathogens in a biosafety cabinet. The Complex800_OBL_V4_DSP protocol enables manual lysis to be performed in a similar way as for the Complex800_V6_DSP protocol. Pretreated samples are transferred to the QIASymphony SP and processed with the Complex800_OBL_V4_DSP protocol.

Note: The Complex800_OBL_V4_DSP protocol requires Buffer ACL and Buffer ATL (ATL). Buffer ACL (cat. no. 939017) and Buffer ATL (ATL) (cat. no. 939016) are not part of the QIASymphony DSP Virus/Pathogen Midi Kit and must be ordered separately.

Manual lysis

1. Pipet 80 µl proteinase K, 295 µl Buffer ATL (ATL), 120 µl Carrier RNA Internal Control Mixture, and 560 µl Buffer ACL into a 4.5 ml tube (Nunc® CryoTube 12.5 x 92 mm, 4.5 ml polypropylene tube, Nunc cat. no. 363452).

Note: When more than one sample will be processed using manual lysis, a stock solution of this solution can be prepared. Simply multiply the volumes required for one sample by the total number of samples to be processed, and include additional volume to the equivalent of 2 extra samples. Invert the tube several times to mix, transfer 1055 µl to a 4.5 ml tube for each sample, and then continue for each sample with step 4.

2. Close the lid and mix by inverting the tube 5 times.
3. Briefly centrifuge the tube to remove droplets from inside the lid.
4. Add 800 µl sample to the tube, close the lid, and mix by pulse-vortexing for 10 s.
5. Incubate the tube at 68°C for 15 min.
6. Briefly centrifuge the tube to remove droplets from inside the lid.
7. Place the inserts for the appropriate sample tubes into a tube carrier and load the sample tubes (without lids).

Preparation of sample material

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

Note: Sample stability highly depends on various factors and relates to the specific downstream application. It has been established for the QIASymphony DSP Virus/Pathogen 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.

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/kit shall be followed during sample preparation, storage, transport, and general handling.

Urine

Urine can be stored at 2–8°C for up to 6 hours. For longer storage, we recommend freezing at –20°C or –80°C. Urine can be processed without further pretreatment. The system is optimized for pure urine samples that do not contain preservatives. To increase sensitivity for bacterial pathogens, samples can be centrifuged. After discarding the supernatant the pellet can be resuspended in at least 800 µl Buffer ATL (ATL) (cat. no. 939016). Use 800 µl of the pre-treated material as sample for preparation of the off-board lysis.

Isolation of genomic DNA from Gram-positive bacteria

DNA purification can be improved for some Gram-positive bacteria by enzymatic pretreatment before transferring the sample to the QIAasymphony SP and starting the Complex800_OBL_V4_DSP protocol.

1. Pellet bacteria by centrifugation at 5000 x *g* for 10 min.
2. Suspend the bacterial pellet in 800 µl of the appropriate enzyme solution (20 mg/ml lysozyme or 200 µg/ml lysostaphin; 20 mM Tris-HCl, pH 8.0; 2 mM EDTA; 1.2% Triton X-100).
3. Incubate at 37°C for at least 30 min.
4. Briefly centrifuge the tube to remove drops from the inside of the lid.
5. Use 800 µl of the pre-treated material as sample for preparation of the off-board lysis.

Viscous or mucous samples

Some samples may be viscous and require liquefaction to enable pipetting. Low-viscosity samples do not require additional preparation. Medium- to high-viscosity samples should be prepared as follows:

1. Dilute the sample 1:1 with 0.3% (w/v) dithiothreitol (DTT).
Note: The 0.3% DTT solution can be made in advance, and stored at –20°C in appropriate aliquots. Thawed aliquots should be discarded after use.
2. Incubate at 37°C until the sample viscosity is suitable for pipetting.
3. Use 800 µl of the pre-treated material as sample for preparation of the off-board lysis.

Dried body fluid and secretion swabs

1. Submerge the dried swab tip in 1050 µl Buffer ATL (ATL) (cat. no. 939016), and incubate at 56°C for 15 min, with continuous mixing. If mixing is not possible, vortex before and after incubation for at least 10 s.
2. Remove the swab and squeeze out all the liquid by pressing the swab against the inside of the tube.
3. Use 800 µl of the pre-treated material as sample for preparation of the off-board lysis.
Note: This protocol is optimized for cotton or polyethylene swabs. When using other swabs, it may be necessary to adjust the volume of Buffer ATL (ATL) to ensure that at least 800 µl is available as sample material.

Respiratory or urogenital swabs

Urogenital swabs (in transport media, e.g., PreservCyt, UTM, eNAT) and respiratory swabs (dried swabs or in transport media, e.g., UTM, eNAT) can be stored at 2–8°C for up to 6 hours. For longer storage, we recommend freezing at –20°C or –80°C.

Storage media for respiratory or urogenital swabs can be used without pretreatment. If the swab has not been removed, press the swab against the side of the tube to squeeze out the liquid. Any excess mucus in the specimen should be removed at this time by collecting it on the swab. Any residual liquid from the mucus and the swab should then be squeezed out by pressing the swab against the side of the tube. Finally, the swab and the mucus should be removed and discarded. If samples are viscous, perform a liquefaction step (see “Viscous or mucous samples” section) before transferring the sample to the QIA Symphony SP. If there is not sufficient starting material, pipet Buffer ATL (ATL) into the transport medium to adjust the required minimum starting volume and vortex the sample for 15–30 seconds in the tube (if the transport medium contains the swab perform this step before removing the swab). Use 800 µl of the material as sample for preparation of the off-board lysis.

Limitations and interfering substances

No significant negative impact of potential interfering substances was observed (for details see the application Performance Characteristics document that can be found under the resource tab of the product page on www.qiagen.com).

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 QIA Symphony DSP Virus/Pathogen Kits.





Storage of eluates

Note: Eluate stability highly depends on various factors and relates to the specific downstream application. It has been established for the QIA Symphony DSP Virus/Pathogen 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.

For short-term storage of up to 24 hours, we recommend storing purified nucleic acids at 2–8°C. For long-term storage of over 24 hours, we recommend storage at –20°C.

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
	This product fulfills the requirements of the European Regulation 2017/746 for in vitro diagnostic medical devices.
	In vitro diagnostic medical device
	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	<p data-bbox="571 336 788 361">Version 2, Revision 1</p> <ul data-bbox="619 378 1257 555" style="list-style-type: none"><li data-bbox="619 378 1118 404">• Update to version 2 for compliance to IVDR<li data-bbox="619 417 1198 442">• Extension of Preparation of sample material section<li data-bbox="619 455 1257 480">• Addition of Limitations and interfering substances section<li data-bbox="619 493 1054 519">• Addition of Storage of eluates section<li data-bbox="619 532 954 557">• Addition of Symbols section

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