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QIAsymphony® SP Protocol Sheet

Complex800_OBL_V4_DSP protocol

This document is the Complex800_OBL_V4_DSP *QIAsymphony SP Protocol Sheet*, R2, for QIAsymphony DSP Virus/Pathogen Midi Kit, version 1.

General information

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

Kit	QIAsymphony 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	Elution volume: 60 µl, 85 µl, 110 µl
Required software version	Version 4.0 or higher

"Sample" drawer

Sample type	Respiratory samples (BAL, dried swabs, transport media, aspirates, sputum) and urogenital samples (urine, transport media)
Sample volume	Depends on type of sample tube used; for more information see **www.qiagen.com/goto/dsphandbooks**
Primary sample tubes	See **www.qiagen.com/goto/dsphandbooks** for more information
Secondary sample tubes	See **www.qiagen.com/goto/dsphandbooks** for more information
Inserts	Depends on type of sample tube used; for more information see **www.qiagen.com/goto/dsphandbooks**
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)	See **www.qiagen.com/goto/dsphandbooks** for more information

Required 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 reagent cartridge.

§ 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.

Selected elution volume

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

* The elution volume selected in 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 **www.qiagen.com/goto/dsphandbooks** 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 material safety data sheets (MSDSs), available from the product supplier.

The QIAsymphony Complex protocols consist of 4 steps: lyse, bind, wash, 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 seconds.
5. Incubate the tube at 68°C for 15 minutes (± 1 minute).
6. Briefly centrifuge the tube to remove droplets from inside the lid.Place the inserts for the appropriate sample tubes into a tube carrier and load the sample tubes (without lids).

Preparation of sample material

Urine

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 QIAsymphony SP and starting the Complex800_OBL_V4_DSP protocol.

1. Pellet bacteria by centrifugation at 5000 x *g* for 10 minutes.
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 minutes (± 2 minutes).
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 (e.g., sputum, respiratory aspirates) 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 Sputasol*† (Oxoid, cat. no. SR0233) or 0.3% (w/v) DTT.

   Note: The 0.3 % DTT solution can be made in advance, and stored at –20°C in appropriate aliquots. A thawed aliquot 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 minutes (± 1 minute), with continuous mixing. If mixing is not possible, vortex before and after incubation for at least 10 seconds.
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

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 mucous in the specimen should be removed at this time by collecting it on the swab. Any residual liquid from the mucous and the swab should then be squeezed out by pressing the swab against the side of the tube. Finally, the swab and the mucous should be removed and discarded. If samples are viscous, perform a liquefaction step (see "Viscous or mucous samples" above) before transferring the sample to the QIAsymphony 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.

* Sputasol (Oxoid, cat. no. SR0233, **www.oxoid.com**) or dithiothreitol (DTT).
† This is not a complete list of suppliers.

Revision history

Document revision history	
R2 12/2017	Update for QIAsymphony Software version 5.0

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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