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QIAsymphony® SP Protocol Sheet

Complex800_V6_DSP protocol

This document is the Complex800_V6_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_V6_DSP
Default Assay Control Set	ACS_Complex800_V6_DSP_default_IC
Editable	Eluate 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	Buffer ATL (ATL)
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

"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†‡	34	60	86	112
Disposable filter-tips, 1500 µl†‡	123	205	295	385
Sample prep cartridges§	18	36	54	72
8-Rod Covers¶	3	6	9	12

* Use of more than one internal control per batch and performing more than one inventory scan requires additional disposable filter-tips. 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 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.

Tubes containing internal control–carrier RNA (CARRIER)–Buffer AVE (AVE) mixture are placed in a tube carrier. The tube carrier containing the internal control–carrier RNA (CARRIER)–Buffer AVE (AVE) mixture(s) must be placed in slot A of the sample drawer.

Depending on the number of samples to be processed, we recommend using 2 ml tubes (Sarstedt, cat. no. 72.693 or 72.694) or 14 ml 17 x 100 mm polystyrene, round-bottom tubes (Becton Dickinson, cat. no. 352051) for diluting the internal control, as described in the table below. The volume can be split into 2 or more tubes.

Calculating the volume of internal control mixture

Tube type	Name on QIAsymphony touchscreen	Calculation of internal control–carrier RNA (CARRIER)–Buffer AVE (AVE) mixture volume per tube
Microtube 2 ml with cap; microtube 2 ml, PP, SKIRTED, (Sarstedt, cat. no. 72.694)	SAR#72.694 T2.0 ScrewSkirt	(n x 120 µl) + 360 µl*
Microtube 2 ml with cap; microtube 2 ml, PP, NON-SKIRTED, (Sarstedt, cat. no. 72.693)	SAR#72.693 T2.0 Screw	(n x 120 µl) + 360 µl*
Tube 14 ml, 17 x 100 mm polystyrene round-bottom (Becton Dickinson, cat. no. 352051)	BD#352051 FalconPP 17x100	(n x 120 µl) + 600 µl†

* Use this equation to calculate the required volume of internal control mixture (n = number of samples; 120 µl = volume of internal control–carrier RNA (CARRIER)–Buffer AVE (AVE) mixture; 360 µl = void volume required per tube). For example, for 12 samples (n = 12): (12 x 120 µl) + 360 µl = 1800 µl. Do not fill the tube with more than 1.9 ml (i.e., a maximum of 12 samples per tube). If more than 12 samples will be processed, use additional tubes, ensuring that the void volume is added per tube.

† Use this equation to calculate the required volume of internal control–carrier RNA (CARRIER)–Buffer AVE (AVE) mixture (n = number of samples; 120 µl = volume of internal control–carrier RNA (CARRIER)–Buffer AVE (AVE) mixture; 600 µl = void volume required per tube). For example, for 96 samples (n = 96): (96 x 120 µl) + 600 µl = 12120 µl.

See **www.qiagen.com/goto/dsphandbooks** for required inserts.

Using FIX labware

Using liquid-level detection (LLD) for sample transfer allows the use of primary and secondary tubes. However, this requires certain dead volumes in the respective tubes. In order to minimize dead volumes, secondary tubes should be used without liquid-level detection. Specific FIX labware is available (e.g., SAR_FIX_#72.694 T2.0 ScrewSkirt) which can also be selected on the touchscreen of the QIAsymphony SP. This tube/rack type imposes aspiration restrictions. The sample is aspirated at a particular height in the tube that is defined by the volume of sample to be transferred. Therefore, it is essential to make sure that the volume listed in the labware list is used. Labware lists are available for download from **www.qiagen.com/goto/dsphandbooks**.

Sample tubes that can be used with or without liquid-level detection and required sample volumes are listed at **www.qiagen.com/goto/dsphandbooks**. Do not use volumes greater or lower than the required volume since this may lead to errors during sample preparation.

Tubes for liquid-level detection and tubes that are not for liquid-level detection can be processed within one batch/run.

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 material safety data sheets (MSDSs), available from the product supplier.

Urine

Urine can be processed without further pretreatment. Transfer the sample to a 2 ml Sarstedt tube (cat. no. 72.693 or 72.694) and place the sample into the tube carrier. Alternatively primary tubes can be used. The required minimum starting volume may vary, depending on the primary tube used. Compatible primary and secondary tube formats, including minimum starting volume required for each protocol, are listed at **www.qiagen.com/goto/dsphandbooks**. 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). Transfer the sample to a 2 ml Sarstedt tube (cat. no. 72.693 or 72.694). Place the sample into the tube carrier and process the sample using the Complex800_V6_DSP protocol and the required FIX labware.

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_V6_DSP protocol.

1. Pellet bacteria by centrifugation at 5000 x *g* for 10 minutes.
2. Suspend the bacterial pellet in 900 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. Transfer the sample to a 2 ml Sarstedt tube (cat. no. 72.693 or 72.694), place the sample in the tube carrier, and continue with the Complex800_V6_DSP protocol and the required FIX labware.

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% (w/v) DTT solution can be made in advance and stored in aliquots at –20°C. Discard thawed aliquots after use.
2. Incubate at 37°C until the sample viscosity is suitable for pipetting.
3. Transfer at least 900 µl of the sample to a 2 ml Sarstedt tube (cat. no. 72.693 or 72.694). Process the sample using the Complex800_V6_DSP protocol.

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

Dried body fluid and secretion swabs

1. Submerge the dried swab tip in 1150 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. Transfer at least 900 µl of the sample to a 2 ml Sarstedt tube (cat. no. 72.693 or 72.694). Process the sample with the Complex800_V6_DSP protocol.

   Note: This protocol is optimized for cotton or polyethylene swabs. When using other swabs, it might be necessary to adjust the volume of Buffer ATL (ATL) to ensure that at least 900 µl is available as sample material.

Respiratory or urogenital swabs stored in transport media

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). Transfer the sample to a 2 ml Sarstedt tube (cat. no. 72.693 or 72.694) and place the sample in the tube carrier. Alternatively, primary tubes can be used. The required minimum starting volume may vary, depending on the primary tube used. Compatible primary and secondary tubes, including minimum starting volume required for each protocol, are listed at **www.qiagen.com/goto/dsphandbooks**.

Revision history

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

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