



Supplementary Protocol for User Self-Validation

QlAsymphony® DSP Virus/Pathogen Kit

Complex800_V6_DSP protocol; Version 2

This protocol is for purification of viral DNA and RNA as well as bacterial DNA from respiratory samples like bronchoalveolar lavage (BAL), sputum, and aspirates using the QIAsymphony SP and the QIAsymphony DSP Virus/Pathogen Midi Kit (cat. no. 937055).

Note: It is the user's responsibility to validate performance using this combination for any procedures used in their laboratory according to local requirements, laws, and regulations.

Equipment and reagents to be supplied by the user

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.

Consumables

- Sample Prep Cartridges, 8-well (cat. no. 997002)
- 8-Rod Covers (cat. no. 997004)
- Filter-Tips, 200 and 1500 µl (cat. no. 990332 and 997024)
- Sample tubes and elution formats (see the labware list which can be found under the resource tab of the product page on www.qiagen.com).
- Vortexer

Additional reagents

Buffer ATL (cat. no. 939016)

For using internal controls

• See the labware list that can be found under the resource tab of the product page on www.giagen.com.

Equipment*

QlAsymphony SP (cat. no. 9001297)

^{*} Prior to use, ensure that instruments have been checked and calibrated according to the manufacturer's recommendations

Labware

 Next to this Supplementary Protocol for User Self-Validation, the labware list can be found under the resource tab of the product page on www.qiagen.com.

Warnings and precautions

Read all instructions carefully before using the kit.

Please be aware of following remaining risk:

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 result 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. Specimens and samples are potentially infectious and must be treated as biohazardous materials.
- Discard sample and assay waste according to your local safety procedures.

CAUTION



DO NOT add bleach or acidic solutions directly to the sample preparation waste.

Buffers in the reagent cartridge (RC) contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

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 Virus/Pathogen Kits.

QSB1



Contains: guanidine thiocyanate and isopropanol. Danger! Flammable liquid and vapor. May be harmful if swallowed or in contact with skin. May be harmful if swallowed and enters airways. Causes severe skin burns and eye damage. May cause drowsiness or dizziness. Harmful to aquatic life with long lasting effects. Contact with acids liberates very toxic gas. Keep away from heat/sparks/open flames/hot surfaces. No smoking. Wear protective gloves/protective clothing/eye protection/face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF exposed or concerned: Immediately call a POISON CENTER or doctor/physician. Rinse mouth. Do NOT induce vomiting. Wash contaminated clothing before reuse. Store in a well-ventilated place. Store locked up. Dispose of contents/container to an approved waste disposal plant.

QSL2



Contains: guanidine thiocyanate. Danger! Harmful if swallowed. May be harmful in contact with skin or if inhaled. Causes severe skin burns and eye damage. Harmful to aquatic life with long lasting effects. Contact with acids liberates very toxic gas. Wear protective gloves/protective clothing/eye protection/face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor/physician.

OSW1



Contains: ethanol, guanidine hydrochloride, and lithium chloride. Warning! Flammable liquid and vapor. May be harmful if swallowed or if inhaled. Causes skin irritation. Causes serious eye irritation. Keep away from heat/sparks/open flames/hot surfaces. No smoking. Wear protective gloves/protective clothing/eye protection/face protection. Call a POISON CENTER or doctor/physician if you feel unwell. Take off contaminated clothing and wash before reuse. Store in a well-ventilated place. Dispose of contents/container to an approved waste disposal plant.

QSW2



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

QSW5



Contains: ethanol and guanidine hydrochloride. Danger! Highly flammable liquid and vapor. May be harmful if swallowed or if inhaled. Causes skin irritation. Causes serious eye irritation. Keep away from heat/sparks/open flames/hot surfaces. No smoking. 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.

General information

Kit	QlAsymphony DSP Virus/Pathogen Midi Kit	
Sample material	Respiratory samples: BAL, sputum, and aspirates	
Protocol name	Complex800_V6_DSP	
Default Assay Control Set	ACS_Complex800_V6_DSP_default_IC	
Editable	Eluate volumes: 60, 85, or 110 µl	
Required software version	Version 4.0 or higher	

"Sample" drawer

Sample type	Respiratory samples: BAL, sputum, and aspirates		
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	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	See the labware list which can be found under the resource tab of the product page on www.qiagen.com for more information.		
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. See the labware list which can be found under the resource tab of the product page on www.qiagen.com for more information.		

"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)

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†‡	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 require additional disposable filter-tips. Use of less than 24 samples per batch decreases the number of disposable tips required per run.

Note: Numbers of filter-tips given may differ from the numbers displayed on the touchscreen depending on settings, for example, number of internal controls used per batch.

Selected elution volume

Selected elution volume (µI)*	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.

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

Selected elution volume (µl)	Volume stock carrier RNA (CARRIER) (μΙ)	Volume internal control (µI)*	Volume Buffer AVE (AVE) (µI)	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.

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, 17×100 mm polystyrene, round-bottom tubes (BDTM, cat. no. 352051) for diluting the internal control, as described in the table below. The volume can be split into 2 or more tubes.

[†] 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.

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

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, 17×100 mm polystyrene round-bottom (BD § , 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 μ l = volume of internal control-carrier RNA (CARRIER)-Buffer AVE (AVE) mixture; 360 μ l = void volume required per tube). For example, for 12 samples (n = 12): (12 x 120 μ l) + 360 μ l = 1800 μ l. 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.

For required inserts, see the labware list which can be found under the resource tab of the product page on www.qiagen.com.

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. 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 at www.qiagen.com under the resource tab of the product page.

Sample tubes that can be used with or without liquid-level detection and required sample volumes are also listed in the labware list available at **www.qiagen.com** under the resource tab of the product page. Do not use volumes greater or lower than the required volume because 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.

Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Because RNases are difficult to inactivate and only minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure.

Yields of nucleic acids

Eluates prepared with carrier RNA (CARRIER) may contain much more carrier RNA (CARRIER) than target nucleic acids. We recommend using quantitative amplification methods to determine yields.

[†] 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 = 12 120 μl

[§] BD was the previous supplier of this tube and Corning Inc. is now the new supplier.

Storing nucleic acids

Note: Eluate 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 short-term storage of up to 24 hours, we recommend storing purified nucleic acids at $2-8^{\circ}$ C. For long-term storage of over 24 hours, we recommend storage at -20° C.

Reagent storage and handling

Attention should be paid to expiration dates and storage conditions printed on the box and labels of all components.

Do not use expired or incorrectly stored components. The QIAsymphony DSP Virus/Pathogen Kits should be stored upright at room temperature (15–25°C). The magnetic particles in the reagent cartridges (RC) remain active when stored at this temperature. Do not store reagent cartridges (RC) at temperatures below 15°C.

Store lyophilized carrier RNA (CARRIER) and Buffer AVE (AVE) at room temperature.

The QIAsymphony DSP Virus/Pathogen Kits contain 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 Virus/Pathogen Kit box displays the expiration date of the kit. The result file documents the expiration dates for only the reagent cartridge (RC) and Buffer ATL (if required).

In-use stability

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

Running batches with low sample numbers (<24) will potentially reduce the total number of sample preparations possible per cartridge.

To avoid reagent evaporation, the reagent cartridge (RC) 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.

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

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.

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 is the responsibility of the user to validate the whole workflow to establish appropriate storage conditions.

Viscous or mucous samples

Some samples (e.g., sputum and 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:

- Dilute the sample 1:1 with Sputasol*† (Oxoid, cat. no. SR0233) or 0.3% (w/v) dithiothreitol (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.

Purification protocol

Important points

- Ensure 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.
- Ensure you are familiar with the supplementary protocol. In particular, note the initial elution volumes required for accurate calculation of internal controls and titer, as well as instructions for calculating the volume of internal control mixture according to the type of tube used.
- Before using a reagent cartridge (RC) for the first time, check that Buffers QSL2 and QSB1 do not contain a precipitate. If necessary, remove the troughs containing Buffers QSL2 and QSB1 from the reagent cartridge (RC) and incubate for 30 minutes at 37°C with occasional shaking to dissolve precipitate. Make sure to replace the troughs in the correct positions. If the reagent cartridge (RC) is already pierced, make sure that the troughs are sealed with Reuse Seal Strips and incubate the complete reagent cartridge (RC) for 30 minutes at 37°C with occasional shaking in a water bath.[‡]
- Try to avoid vigorous shaking of the reagent cartridge (RC) otherwise foam may be generated, which can lead to liquid-level detection problems.

^{*} Sputasol (Oxoid, cat. no. SR0233, www.oxoid.com) or dithiothreitol (DTT).

[†] This is not a complete list of suppliers.

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

Before starting a protocol, check whether precipitate has formed in Buffer ATL. If necessary, dissolve 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

- Prepare all required mixtures, including mixtures containing carrier RNA (CARRIER) and internal controls (optional) just before starting. For more information, see section "Preparation of internal control-carrier RNA (CARRIER)-Buffer AVE (AVE) mixture", page 5.
- Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex the trough containing the magnetic particles vigorously for at least 3 minutes before first use.
- Before loading the reagent cartridge (RC), remove the cover from the trough containing the magnetic particles and open the enzyme tubes. Make sure that the enzyme has been equilibrated to room temperature (15–25°C).
- Make sure that the piercing lid (PL) is placed on the reagent cartridge (RC) or, if using a partially used reagent cartridge, make sure
 the Reuse Seal Strips have been removed.
- 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.
- For information about sample tubes see the labware list which can be found under the resource tab of the product page on www.qiagen.com).

Procedure

- 1. Close all drawers and the hood.
- 2. Switch 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 in to the instrument.
- 4. Ensure that the "Waste" drawer is prepared properly and perform an inventory scan of the "Waste" drawer, including the tip chute and liquid waste. Replace the tip disposal bag if necessary.
- 5. Load the required elution rack into the "Eluate" drawer. For more information concerning the required elution rack, see the labware list that can be found under the resource tab of the product page on **www.qiagen.com**. Use only "Elution slot 1" with the corresponding cooling adapter. 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. Load the required reagent cartridge(s) (RC) and consumables into the "Reagents and Consumables" drawer.
- 6. To load Buffer ATL, press the R+C button in the touchscreen to open the screen that shows the consumables status ("Consumables/8-Rod Covers/Tubes/Filter-Tips/Reagent Cartridges"). Press the Scan Bottle button to scan the bar code of the bottle of Buffer ATL with the handheld bar code scanner. Press OK.
 - Ensure that the bottle of Buffer ATL is scanned, opened, and placed into the position specified on the touchscreen before starting the inventory scan. Otherwise, the inventory scan must be repeated after scanning, opening, and placing the bottle of Buffer ATL 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. Place the tube(s) containing the carrier RNA (CARRIER)-Buffer AVE (AVE) mixture (including optional internal control) into the tube carrier and load into slot A of the "Sample" drawer.
- 10. Using the touchscreen, enter the required information for each batch of samples to be processed. Enter the following information:
 - O Sample information (depending on sample racks used)
 - O Protocol to be run ("Assay Control Set")
 - O Elution volume and output position
 - O Tubes containing the carrier RNA (CARRIER)-Buffer AVE (AVE) mixture (including optional internal control)

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.

- 11. 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".
- 12. Retrieve the elution rack containing the purified nucleic acids from the "Eluate" drawer. 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 experience condensation or evaporation. Result files are generated for each elution plate.

Note: As eluate volumes might vary, ensure that the correct eluate volume is used for the downstream application. The nucleic acid is ready to use or can be stored at $2-8^{\circ}$ C or -20° C.

- 13. If a reagent cartridge (RC) is only partially used, seal it with the provided Reuse Seal Strips and close tubes containing proteinase K with screw caps immediately after the end of the protocol run to avoid evaporation. Close the Buffer ATL bottle and store at 15–25°C.
- 14. Discard used sample tubes, plates, and waste according to your local safety regulations.
- 15. 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.
- 16. Close the instrument drawers, and switch off the QIAsymphony SP.

Document Revision History

Date	Changes
09/2022	Version 2, Revision 1
	Creation of document

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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