

QIASymphony SP Workflow Guide

Pathogen complex off-board lysis protocols for purification of viral DNA, viral RNA, and bacterial DNA

This document provides information about simultaneous purification of viral DNA, viral RNA, and bacterial DNA from primary sample material using the Complex200_OBL_V1, Complex400_OBL_V1, and Complex800_OBL_V1 protocols in combination with the QIASymphony® Virus/Bacteria Kits and the QIASymphony SP with software version 2.1.

The QIASymphony Complex protocols consist of four steps: lyse, bind, wash, elute. For some samples, it is useful to manually perform lysis, for example, for inactivation of pathogens that must be inactivated in a biosafety cabinet. The pathogen complex off-board lysis protocols enable a manual lysis step similar to that included in the pathogen complex protocols. Pretreated samples are transferred to the QIASymphony SP and processed using the pathogen complex off-board lysis protocols. The example workflow described assumes that samples will be loaded using the tube carrier, that reagent cartridges are unopened, and that Assay Control Sets will not be assigned to the samples using the “Fast Setup” button.

IMPORTANT: Before using the QIASymphony SP, it is essential to read the *QIASymphony SP User Manuals* carefully and pay particular attention to the safety information. Be sure to read the *QIASymphony Virus/Bacteria Handbook* and pay particular attention to the “Safety” and “Important Notes” sections.

Note: The protocols require Buffer ACL and Buffer ATL. Buffer ACL and Buffer ATL are not included in the QIASymphony Virus/Bacteria Kits and must be ordered separately (Buffer ACL (200 ml), cat. no. 939015; Buffer ATL (4 x 50 ml), cat. no. 939011).



Manual off-board lysis

Procedure

1. **Pipet proteinase K, Buffer ATL, Carrier RNA Internal Control Mixture, and Buffer ACL into an appropriate tube. Close the lid and mix by inverting the tube 5 times.**

The required volumes of proteinase K and Buffers are given in Table 1 and compatible tubes are listed in Table 2.

For preparation of the Carrier RNA Internal Control Mixture, see the *QIAasympyphony Virus/Bacteria Kit Handbook*.

2. **Briefly centrifuge the tube to remove drops from the inside of the lid.**
3. **Add the appropriate sample volume, close the lid, and mix by pulse-vortexing for 10 s.**
The required sample volumes for the respective protocols are given in Table 1.
4. **Incubate at 68°C for 15 min.**
5. **Briefly centrifuge the tube to remove drops from the inside of the lid.**
6. **Place the inserts for the appropriate sample tubes into a tube carrier and load the sample tubes (without lids) into them (see "Automated Procedure", step 9).**

Table 1. Volumes of proteinase K and buffers for off-board lysis protocols

	Complex200_OBL-V1	Complex400_OBL-V1	Complex800_OBL-V1
Proteinase K	20 μ l	40 μ l	80 μ l
Buffer ATL	100 μ l	165 μ l	295 μ l
Carrier RNA Internal Control Mixture	120 μ l	120 μ l	120 μ l
Buffer ACL	190 μ l	315 μ l	560 μ l
Sample	200 μ l	400 μ l	800 μ l

Table 2. Sample tubes for use in combination with pathogen complex off-board lysis protocols

Material	Supplier	Cat. no.	Complex 200_OBL-V1	Complex 400_OBL-V1	Complex 800_OBL-V1
Microtube 2ml with cap; Microtube 2ml PP, non- skirted	Sarstedt	72.693	+	+	-
Microtube 2ml with cap; Microtube 2ml PP, skirted	Sarstedt	72694	+	+	-
Nunc CryoTube™ 12.5 x 92 (4.5 ml)	Nunc	363452	+	+	+
Reagent and centrifuge tube 16.8 x 67	Sarstedt	55533	+	+	+
Reagent and centrifuge tube 16.8 x 67	Sarstedt	55524	+	+	+
17 x 100 mm, 14 ml polystyrene round- bottom test tube	BD	352051	+	+	+

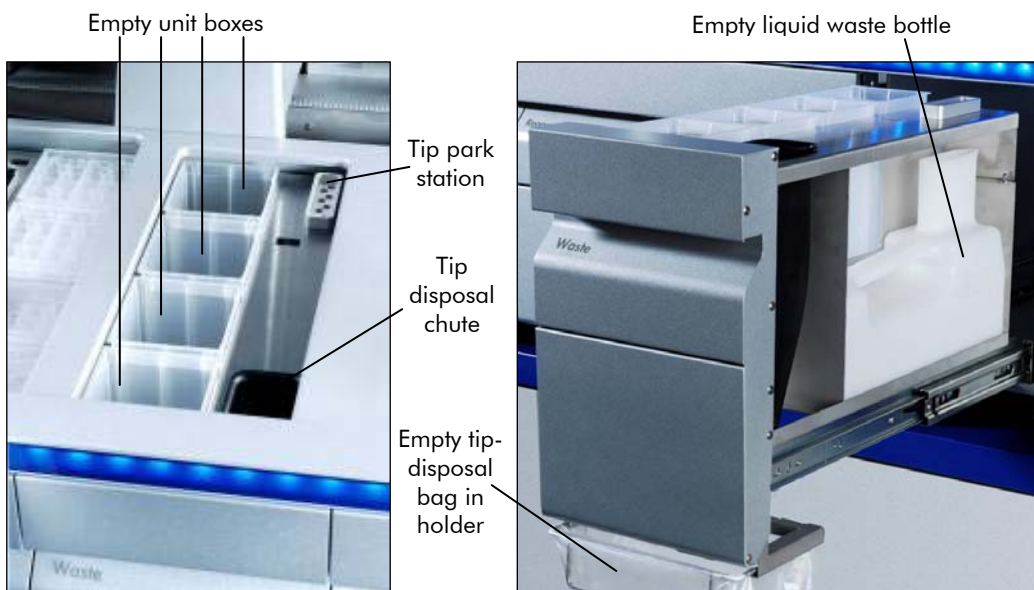
For additional information about sample tubes, visit www.qiagen.com/goto/QIASymphony.

Automated protocol

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 is complete.
3. Log into the instrument (drawers will unlock).
4. Open the "Waste" drawer and load it as shown below.

Note: If using empty 8-Rod Cover unit boxes as waste unit boxes ensure that spacers are removed before loading into the "Waste" drawer.



5. Close the "Waste" drawer and start the inventory scan. A "clicking" noise indicates that the scan has finished and the "Stop Scan" button is no longer displayed.

6. Open the "Eluate" drawer and perform the following actions:

- Using the touchscreen, choose the elution slot to be used. Alternatively, scan the bar code of the elution slot to be used with the handheld bar code scanner.
- Press the "Yes" button.
- If using elution microtubes (EMTR) for elution, scan the bar code with the handheld bar code scanner.

If using any other type of elution rack, select the appropriate type of elution rack from the list displayed in the touchscreen.

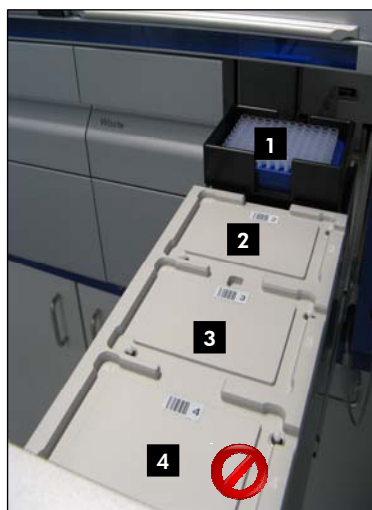
Optional: Enter an elution rack ID and press "OK".

- Place the elution rack onto the chosen elution slot. We recommend using "Elution slot 1", which is cooled, with the appropriate cooling adapter for the selected elution rack. If "Elution Slots 2-4" are used, the samples will be marked as unclear in the result file.

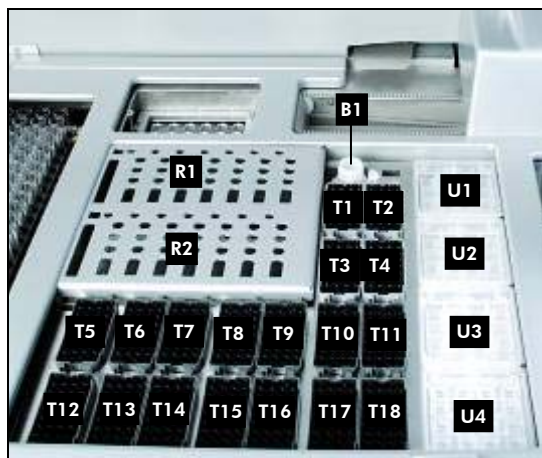
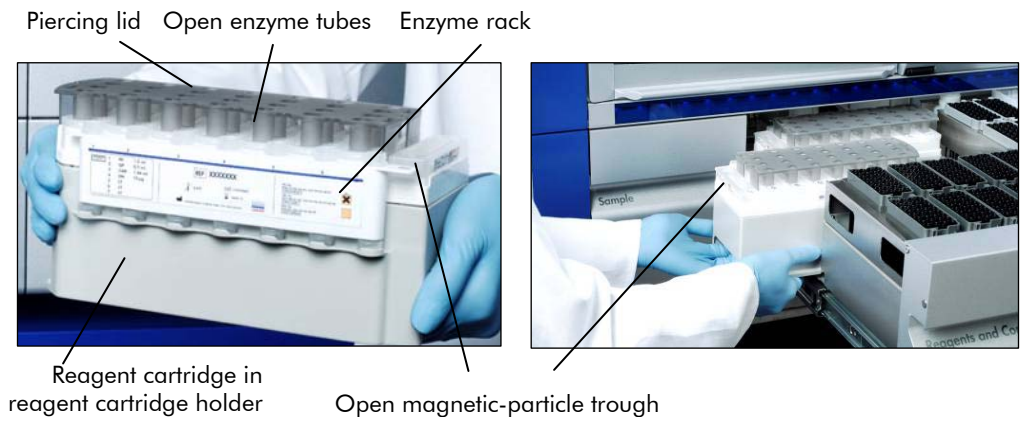
Important: Do not place an elution rack with more than 24 wells in "Elution slot 4".

- Press the "Add" button.
- Close the "Eluate" drawer, and press "OK" to start the inventory scan. A "clicking" noise indicates that the scan has finished and the "Stop Scan" button is no longer displayed.
- Press the "Close" button.

Note: To use an elution microtube rack on the cooling position ("Elution slot 1"), remove the bottom plate of the elution microtube rack and use the cooling adapter.



7. Open the "Reagents and Consumables" drawer and load it as shown below.



- | | | | |
|------------------------|---|-----------------------|--------------------------------------|
| R1 R2 | Reagent cartridges from the QIASymphony Virus/Bacteria Mini or Midi Kit | U1 – U3 | Sample prep cartridges in unit boxes |
| T1 – T4 | 200 μ l filter-tips | U4 | 8-Rod Covers in unit box |
| T5 – T18 | 1500 μ l filter-tips | B1 | Buffer ATL bottle |

Note: Ensure the correct reagent cartridge is used. For the Complex200_OBL_V1 protocol, use the reagent cartridge provided with the QIASymphony Virus/Bacteria Mini Kit; for the Complex400_OBL_V1 and Complex800_OBL_V1 protocols, use the reagent cartridge provided with the QIASymphony Virus/Bacteria Midi Kit.

Note: Ensure that the correct enzyme rack is placed into the reagent cartridge holder, even if proteinase K is added manually during preparation of the off-board lysis. If only off-board lysis protocols are used in a run, it is not necessary to remove the caps from the tubes. If protocols other than off-board lysis protocols are used, be sure to remove the caps from the tubes.

Note: Ensure that the magnetic particles in the magnetic-particle trough are fully resuspended. Remove the magnetic-particle trough from the reagent cartridge frame and vortex it vigorously for at least 3 minutes. Replace the trough in the reagent cartridge frame and make sure to remove the cover of the magnetic-particle trough.

Note: If only off-board lysis protocols are used in a run, there is no need to scan and place a Buffer ATL bottle in position B1 before starting the inventory scan since Buffer ATL was added during the manual procedure.

8. Close the “Reagents and Consumables” drawer and start the inventory scan. The inventory scan will run for some time. A “clicking” noise indicates that the scan has finished and the “Stop Scan” button is no longer displayed.

If any problems occur during the inventory scan, check the loading of the “Reagents and Consumables” drawer. You will be asked to perform another inventory scan after opening and closing the drawer.

9. Load the samples using the tube carrier.

- If required, place the inserts for the appropriate sample tubes into a tube carrier and load the sample tubes into them. If sample tubes are not bar code labeled, do not use different types of inserts within the same batch.

- To avoid damaging the QIASymphony SP, only use tubes that are recommended by QIAGEN. Detailed information about recommended tubes is available at www.qiagen.com/goto/QIASymphony.



- Ensure that samples have been thoroughly mixed and that tubes contain sufficient volumes for the protocol. Detailed information about recommended tubes and volumes is available at www.qiagen.com/goto/QIASymphony.

- Open the “Sample” drawer.

- Place the tube carrier onto the stop line of the appropriate slot and wait until the green LED starts to flash.

- Slide the carrier into the slot, steadily and continuously. Push the carrier until it will not slide further and the entire carrier has passed over the stop line.

Note: After successful loading, the color of the LED changes to orange. If it is red, remove the rack and reload it.

- Close the “Sample” drawer.

10. Press the “Batch 1” button in the touchscreen. The color of the button is now blue and the batch status is “LOADED”.

11. Assign the correct type of labware:

- The default tube for the type of insert used will be displayed in the touchscreen. If using a tube other than the default, this must be changed in the QIAAsymphony software.
Note: The default tube type for a type of insert can be changed by the supervisor within the configuration menu.
- If using sample tubes that are not the default for the insert, select the corresponding sample positions, and then select the type of tube the samples are in from the drop-down list at the right-hand side of the panel.
- To edit the sample IDs, press the "ID/Type" button in the command bar. Choose the appropriate sample, press the "Edit ID" button, enter the sample ID, and press "OK". Every sample must be assigned a tube type and an ID number before continuing with the next step.
- Press the "Next" button.

12. Assign an Assay Control Set:

- Select the samples to be processed with one Assay Control Set.
- Select the "Pathogen" application and select an Assay Control Set.
- Repeat the process until all samples have been assigned the correct Assay Control Set.
Note: Each internal control used needs a separate Assay Control Set. Assay Control Sets are created with the QIAAsymphony Management Console and should be transferred to the QIAAsymphony SP before assigning them to samples.

QIAAsymphony SP protocol	Sample volume	QIAAsymphony	
		Virus/Bacteria Kit	Assay Control Set
Complex200_OBL_V1	200 µl	Mini	ACS_Complex200_OffBoardLysis_V1
Complex400_OBL_V1	400 µl	Midi	ACS_Complex400_OffBoardLysis_V1
Complex800_OBL_V1	800 µl	Midi	ACS_Complex800_OffBoardLysis_V1

- Press the "Next" button.

13. Assign the elution rack and elution volume:

- Select the elution slot.
- Select the elution volume from the drop-down list on the right-hand side of the panel.
- Press the "Queue" button.
Note: The order in which the batches are queued determines the position of the corresponding eluates in the elution rack. We recommend loading and queuing batches in sequential order to ensure correct eluate positioning.

14. Load internal controls:

- No internal control tubes are required as internal controls and carrier RNA are added during manual preparation of lysate.

15. **Press the “Run” button to start the purification procedure.**

Important: We strongly recommend performing a new inventory scan of the “Reagents and Consumables” drawer after running 12 batches.

Tips and tricks

Comments

Samples

To ensure reliable sample transfer, avoid generating foam in sample tubes. Ensure that the samples have been thoroughly mixed before transferring to the instrument and that no precipitates are present.

Information about minimum sample volumes for samples in primary and secondary tubes is available at www.qiagen.com/goto/QIASymphony. A list of tubes and plates that can be used for the different protocols is also available. Please refer to this information before loading the samples into the "Sample" drawer to ensure that your samples will be correctly processed.

Inventory scan

Ensure that all tip racks are correctly inserted in the "Reagents and Consumables" drawer. All racks should be level in the drawer.

Storage of unopened reagent cartridges

QIASymphony Virus/Bacteria Kits should be stored at room temperature (15–25°C). Do not store reagent cartridges at temperatures below 15°C. QIASymphony Virus/Bacteria Kits contain ready-to-use proteinase K solution that can be stored at room temperature.

Storage of partially used reagent cartridges

When tightly sealed with a Reuse Seal Set, partially used reagent cartridges can be stored for a maximum of 2 weeks, enabling cost-efficient reuse of reagents and more flexible sample processing. If a reagent cartridge is partially used, seal it with the provided Reuse Seal Set immediately after the end of the protocol run to avoid evaporation.

To avoid evaporation, the reagent cartridge should not be open for more than 15 hours (including run times) at a maximum environmental temperature of 30°C.

Running batches with low sample numbers (<24) will increase both the time that the reagent cartridge is open and the required buffer volumes, potentially reducing the total number of sample preparations possible per cartridge.

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Comments

Reuse of partially used reagent cartridges	We recommend writing the date on the enzyme rack and reagent cartridge with a marker pen before the first use to prevent mix-up with other opened or unopened reagent cartridges.
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Troubleshooting guide

For more detailed troubleshooting information, read the relevant sections of the *QIASymphony SP User Manuals* and the *QIASymphony Virus/Bacteria Handbook*.

Comments

Aborted run

Switch from the main menu to the maintenance screen and press the "Cleanup automatic" button.

Enables automatic worktable cleanup if:

- A batch was aborted due to an error
- A batch was stopped by the user

There are two types of "Automatic" cleanup:

- One specifically for use with investigator protocols (please contact QIAGEN Technical Service for details)
- One for all other protocols. This type of cleanup is divided into three branches ("User interrupted run", "Crash occurred", and "Other") and may also include manual intervention.

Important: After successful cleanup it is necessary to empty all slot positions ("Sample" drawer and "Eluate" drawer) and restart the instrument.

We recommend performing "Automatic" cleanup only if the QIASymphony SP was not switched off after an error occurred, and if no changes were made to the consumables loaded on the worktable.

Note: Only in cases of emergency should the "Automatic" cleanup procedure be used if the instrument was switched off.

Note: Rodslots are the black magnetic rods of the magnetic head (MH).

Important: Do not manually remove any consumables from the worktable unless instructed to do so. Be sure to follow the safety guidelines and instructions given in the "Preventive Maintenance" chapter of the *QIASymphony SP User Manual — General Description*, and dispose of the liquid waste and used consumables according to local safety regulations.

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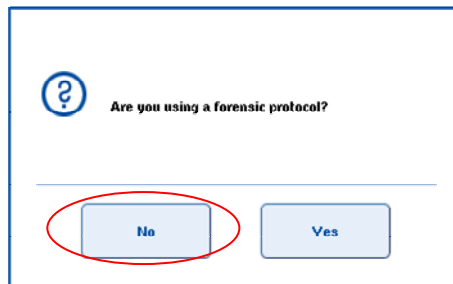
Comments

To perform "Automatic" cleanup, proceed as follows:

Press "Automatic" in the "Maintenance" screen.

The "Welcome" screen appears. Press "OK" to continue.

The following message appears. Press "No".



Select the appropriate branch of the "Automatic" cleanup ("User interrupted run", "Crash occurred", or "Other). See table for more information. Press "OK" to continue.

User interrupted run	Select if the user paused or stopped the run and no error occurred. No manual intervention is required.
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Important: Cannot be used if an inventory scan was performed after the batch was aborted/stopped. Use "Other" instead.

Crash occurred	Select if an error occurred. Cleanup is semi-automatic, some manual intervention is required.
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Other	Select in all other cases not outlined by the "User interrupted run" and "Crash occurred". Cleanup is semi-automatic, some manual intervention is required.
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Comments

Note: This selection will not be available if the system was restarted before. A semi-automatic cleanup with manual intervention will be performed in this case.

Follow the instructions on the screens. They will guide the user step-by-step through the cleanup protocol.

Note: By following the instructions on the screen, all used consumables will be removed from the magnetic head and worktable, and all liquids in sample prep cartridges will be discarded.

After cleanup, empty all slot positions (i.e., in the "Sample" and "Eluate" drawer) and restart the QIASymphony SP.

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Korea (South) = 1544 7145

Sweden = 020-790282

Austria = 0800/281010

France = 01-60-920-930

Luxembourg = 8002 2076

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