

August 2015

QIASymphony[®] SP Protocol Sheet

SP800_HC2_V1_DSP protocol for SurePath[®] specimens

This document contains instructions for preparation of a sample taken from a SurePath specimen that has not been processed using any other diagnostic method, including the BD[™] PrepMate[®] and BD PrepStain[®] Slide Processor.

Intended Use

For in vitro diagnostic use.

This protocol is used with cervical specimens stored in SurePath Preservative Fluid that have not been processed using any other diagnostic method (including the BD PrepMate and BD PrepStain Slide Processor) using QIAasymphony SP and the QIAasymphony DSP HPV Media Kit. Sample extracts prepared with this protocol are ready for testing with the *digene*® HC2 High-Risk HPV DNA Test and may only be tested using the Rapid Capture® System.

Collect specimens in SurePath Preservative Fluid according to the appropriate instructions for use.

Important: Do not use this protocol sheet to process SurePath post-gradient cell pellet samples.

Important: Read *QIAasymphony DSP HPV Media Kit Instructions For Use (Handbook)* and *digene HC2 High-Risk HPV DNA Test Instructions for Use* before using this protocol, paying attention to the safety information provided.

Version management

This document is the *SP800_HC2_V1_DSP protocol for SurePath specimens QIAasymphony SP Protocol Sheet*, HB-1554-S02-003. This document is used in conjunction with the QIAasymphony DSP HPV Media Kit, Version 1.

Overview

Kit	QIAsymphony DSP HPV Media Kit (cat. no. 937358)
Sample material	Liquid-based cytology cervical specimens stored in SurePath Preservative Fluid
Protocol name	SP800_HC2_V1_DSP
Default Assay Control Set	ACS_SP800_HC2_V1_DSP
Sample volume*	950 µl required sample volume 800 µl processed sample volume
Sample extract volume	Approximately 90 µl
Required software version	Version 4.0

* QIAsymphony SP aspirates 800 µl of sample from the bottom of the sample tube. At least 950 µl of sample volume is required for a **valid** flagged sample. Samples with less than 950 µl of sample volume may result in an **invalid** flagged sample. An **invalid** flagged sample is unacceptable for testing with the *digene* HC2 High-Risk HPV DNA Test.

Materials required but not provided

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), available from the product supplier.

Plasticware loaded on QIAsymphony SP

Plasticware	One batch, 24 samples*	Two batches, 48 samples*	Three batches, 72 samples*	Four batches, 88 samples*
Disposable filter-tips, 1500 µl†	63	123	183	227
Sample prep cartridges	6	12	18	22
8-Rod Covers	3	6	9	11

* Performing more than one inventory scan requires additional disposable filter-tips. Use of fewer than 24 samples per batch decreases the number of disposable filter-tips required per run.

† The number of required filter-tips includes filter-tips for one inventory scan per reagent cartridge (RC).

Note: Numbers of filter-tips given may differ from the numbers displayed in the touchscreen depending on settings. We recommend loading the maximum possible number of tips.

Additional materials

Refer to *QIASymphony DSP HPV Media Kit Instructions for Use (Handbook)* for additional information on the following materials.

- 2 ml, blue, non-skirted, polypropylene conical tubes available from QIAGEN (cat. no. 997055)
- 2 ml sample carrier tube insert available from QIAGEN (cat. no. 9242083)
- Hybridization Microplates available from QIAGEN (cat. no. 6000-1203)
- Microplate Lids available from QIAGEN (cat. no. 6000-5001)
- Tip Disposal Bags available from QIAGEN (cat. no. 9013395)
- Cooling Adapter, MTP, RB, Qsym available from QIAGEN (cat. no. 9018085)
- Hybrid Capture® System Microplate Heater I
- 65 ± 2°C waterbath
- Single-channel pipet; variable settings for 20–200 µl and 200–1000 µl volumes
- Disposable aerosol-barrier pipet tips for single-channel pipet (20–200 µl and 200–1000 µl)
- Vortexer

Specimen Handling and Storage

Important: Do not collect a SurePath cervical specimen for sample preparation with the QIASymphony DSP HPV Media Kit if high concentrations of anti-fungal cream, anti-inflammatory cream or contraceptive jelly are present.

Following collection, store SurePath specimens for up to 6 weeks at 5–25°C prior to sample preparation using QIASymphony SP and the QIASymphony DSP HPV Media Kit. The SurePath specimen used must not have been processed using any other diagnostic method, including the BD PrepMate and BD PrepStain Slide Processor. Automated sample preparation requires 950 µl of the SurePath specimen.

Note: Manual sample preparation for the *digene* HC2 High-Risk HPV DNA Test requires a SurePath post-gradient cell pellet sample. Do not use a SurePath post-gradient cell pellet sample for this procedure. Refer to *digene HC2 High-Risk HPV DNA Test Instructions for Use* for additional instructions.

Procedure

Important points before starting

- Refer to the user manuals supplied with QIAasymphony SP for additional instructions on operating the instrument.
- The hybridization microplate prepared with this protocol can only contain sample extracts generated using one QIAasymphony SP protocol. The hybridization microplate cannot contain multiple specimen types or samples prepared using other methods.
- The user must reserve the first column of the hybridization microplate on QIAasymphony SP for the calibrators and quality controls provided with the *digene* HC2 High-Risk HPV DNA Test.
- Do not use a rack ID with QIAasymphony SP that was already used with the *digene* HC2 System Software. If the rack ID already exists in the *digene* HC2 System Software, the *digene* HC2 System Software will prompt for a new input or require the user to cancel the plate map import.
- Do not use a rack ID greater than 20 characters in length or a specimen ID greater than 30 characters in length. If either input is greater than the specified maximum characters, the *digene* HC2 System Software, during plate map import, will prompt for a new input or require the user to cancel the plate map import.
- When creating the sample or rack ID, adhere to the following requirements to ensure compatibility with the *digene* HC2 System Software:
 - Do not use a space before or after the ID
 - Do not use case-sensitive characters to differentiate IDs
 - Only use alphanumeric characters, hyphens and spaces to create an ID
- If using a quality control ID, as defined in the *digene* HC2 System Software, as a sample ID on QIAasymphony SP, the flagging of the sample (such as **valid**, **unclear** or **invalid**) and all comments from QIAasymphony SP will not transfer during the plate map import. The flagging and comments of the QIAasymphony SP processing must be entered manually into the *digene* HC2 System Software.
- If the “Eluate” drawer is opened when a batch is running (e.g., if the hybridization microplate that contains the sample extracts is removed), the run will pause, and the user must perform an inventory scan of the “Eluate” drawer. Make sure to complete the “Eluate” drawer scan before continuing with the protocol.

Things to do before starting

- Allow at least 60 minutes for the Microplate Heater I to equilibrate to 70°C from a cold start. Not allowing time for this warm-up period could result in melting of the hybridization microplate. Refer to *Microplate Heater I User Manual* for additional instructions.
- Make sure that the waterbath is at 65°C ± 2°C and the water level is adequate to immerse the entire volume in the tube.

Loading the “Reagents and Consumables” drawer

1. Close all drawers and the hood.
2. Power ON QIAasympy SP and wait until the initialization procedure has finished.
Note: The power switch is located at the bottom-left corner of QIAasympy SP.
3. Log in to the instrument.
4. Prepare the “Waste” drawer according to the following table.

Make sure that the covers of the unit boxes are removed before loading the unit boxes into the “Waste” drawer. If you are using 8-Rod Cover boxes for collecting used sample prep cartridges and 8-Rod Covers, make sure that the box spacer has been removed.

Unit box holder 1–4	Empty unit boxes
Waste bag holder	Waste bag
Liquid waste bottle holder	Empty liquid waste bottle
Tip chute holder	Tip chute
Tip park station	Empty tip park station

5. Perform an inventory scan of the “Waste” drawer, including the tip chute and liquid waste.
6. Load the required reagent cartridge(s) (RC) and consumables into the “Reagents and Consumables” drawer according to the following table.

Position A1 and/or A2	Reagent cartridge (RC)
Position B1	Empty
Tip rack holder 1–17	Disposable filter-tips, 1500 µl
Unit box holder 1–4	Unit boxes containing sample prep cartridges or 8-Rod Covers

See “Plasticware loaded on QIAasympy SP,” page 3, for more information about the number of consumables to load.

Important: Do not refill tip racks or unit boxes with consumables remaining. QIAAsymphony SP can use partially used tip racks and unit boxes.

Important: Make sure not to touch the disposable filter-tips to the instrument drawer to prevent contamination.

If using new sample prep cartridges or 8-Rod Covers, invert the unit box holders while holding a hand over the opening and slide the materials in the unit box holders up and down to make sure the materials are properly aligned in the unit box holders.

7. Perform an inventory scan of the “Reagents and Consumables” drawer.

Notes:

- Initiate a new scan of the reagent cartridge (RC) after processing 88 samples.
- Avoid scanning the reagent cartridge (RC) in short intervals (e.g., after additional loading of consumables or after running a batch of 24 samples).

Loading the “Eluate” drawer

1. Place the hybridization microplate into the Cooling Adapter, MTP, RB, Qsym with the A1 microplate well aligned with the adapter corner labeled “A1”.

2. Open the “Eluate” drawer.

The **Eluate Drawer/Elution Slot** screen appears.

3. Use the handheld bar code scanner to select slot 1. Alternatively, press the corresponding slot button on the touchscreen.

The **Eluate Drawer/Elution Slot/Change Rack 1** screen appears.

4. Use the handheld bar code scanner to input the rack ID. Alternatively, press the **Rack ID** button and enter the rack ID using the keyboard displayed on the screen.

The entered rack ID is displayed.

5. Place the hybridization microplate with the adapter in the “Eluate” drawer in slot 1 (the cooling position). Make sure the hybridization microplate is oriented with the A1 microplate well in the upper-left corner.

Important: The hybridization microplate with the adapter must be oriented with the A1 microplate well in the upper-left corner for proper use with the *digene* HC2 High-Risk HPV DNA Test and the RCS.

6. In the **Available rack types:** drop-down list, press the **Micro Plate** button.

A list of available microplates displays.

7. Press the **QIA#6000-1203 *MTP96 RB** button from the available microplates listed.

The **Reserved Columns** option becomes available after a rack type has been selected. The first column on the left side of the hybridization microplate must be reserved.

8. Use the **+** and **-** buttons to reserve **1** column of the hybridization microplate.
9. Close the “Eluate” drawer.
10. Press the **OK** button.

QIAasympy SP performs an inventory scan of the “Eluate” drawer. Sample processing pauses, and the robotic arm moves to the “Eluate” drawer to check that the selected elution slots contain an elution rack.

Preparing the samples

Transfer the specified sample volume to a sample tube just before starting the run.

1. For each specimen, label a 2 ml, blue, non-skirted, polypropylene conical tube. Alternately, place a bar code on the tube.
2. In the sample carrier using the 2 ml sample carrier tube insert, place the sample tubes in the order in which they will be placed onto the hybridization microplate. Orient the bar codes to the left so that they can be read by the bar code reader, as applicable.
3. Handling one specimen at a time, mix the SurePath specimen vial with a vortexer for 5–10 seconds.

4. As cells settle quickly, immediately remove the lid of the SurePath specimen vial and pipet 950 µl of specimen into the bottom of the corresponding sample tube in the tube carrier.

Pipetting into the bottom of the sample tube minimizes cellular material adhering to the inside of the sample tube.

Make sure of reliable sample transfer by not generating foam.

5. Replace the lid on the SurePath specimen vial.
6. Repeat for each additional specimen, as applicable.
7. Load the sample carrier in the “Sample” drawer.

Running QIAsymphony SP

1. Using the touchscreen, enter the required information for each batch of samples to be processed.

Enter the following information:

- Sample IDs
- Sample tube type based on the sample carrier tube insert used: **Tube Insert 3B**, then **QIA#997055 T2.0 Conical blue**
- Protocol to be run: **SP800_HC2_V1_DSP**
- Output position: **Elution slot 1**

2. Press **Queue**.

The status of the batch changes from **LOADED** to **QUEUED**. As soon as one batch is queued, the **Run** button appears.

3. Press the **Run** button to start the QIAsymphony SP protocol run.

All steps of the protocol run are fully automated. At the end of the protocol run, the status of the batch changes from **RUNNING** to **COMPLETED**.

Remove the hybridization microplate from the “Eluate” drawer immediately after the protocol run has finished. Depending on temperature and humidity, leaving the hybridization microplate in QIAsymphony SP after the protocol run is complete may cause condensation or evaporation.

4. Open the “Eluate” drawer.

5. On the touchscreen, press **Slot 1** and press **Remove**.

A message appears asking if you want to remove the rack.

6. Press the **Yes** button.

7. Remove the cooling adapter containing the hybridization microplate from the “Eluate” drawer.

Remove the hybridization microplate from the cooling adapter. Cover the hybridization microplate with a microplate lid.

Important: Avoid splashing the sample extracts when handling the hybridization microplate.

8. Press **OK**.

QIAsymphony SP scans the “Eluate” drawer.

Result files are generated for each hybridization microplate.

9. Immediately proceed to “Denaturing the sample extracts.”

Denaturing the sample extracts

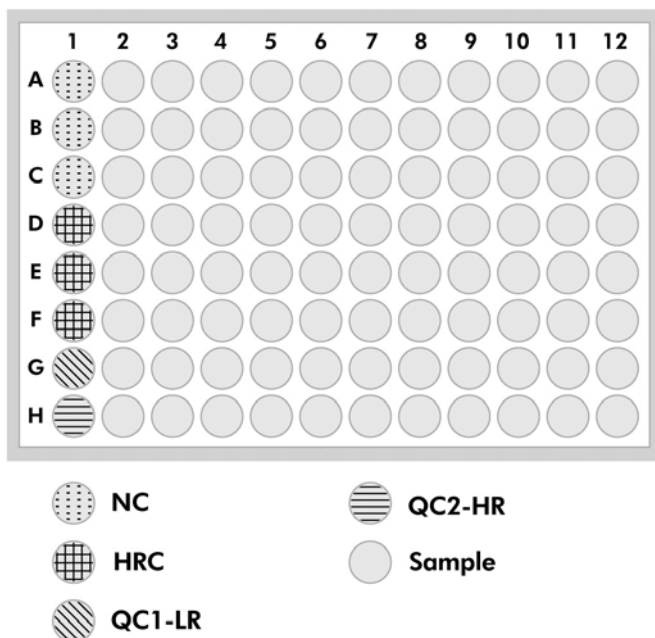
1. Visually inspect the sample extracts. Exclude from further testing any sample extract without a visible amount of magnetic particles.
2. Place the hybridization microplate in the Microplate Heater I equilibrated to $70 \pm 2^{\circ}\text{C}$, making sure not to cause splashing. Incubate the hybridization microplate for 90 ± 5 minutes.
3. During the incubation of the sample extracts, denature the calibrators and quality controls supplied with the *digene* HC2 High-Risk HPV DNA Test.
Refer to *digene HC2 High-Risk HPV DNA Test Instructions for Use* for additional instructions.
4. Perform "Cleaning QIA Symphony SP," page 12, during the incubation of the hybridization microplate.
5. After the incubation, remove the hybridization microplate from the Microplate Heater I.
6. Remove the microplate lid from the hybridization microplate and discard the microplate lid.
7. Place the hybridization microplate on a bench and equilibrate to room temperature for 15 ± 3 minutes.

Adding the denatured calibrators and controls, and storage

The *digene* HC2 System Software requires that the calibrators and quality controls are in the specified and correct location on the microplate in order to verify the assay run. The correct placement of the calibrators and quality controls in the hybridization microplate is essential for valid assay results. The denatured calibrators and quality controls are added into the following positions on the microplate:

- Negative Calibrator (NC) replicates in microplate wells A1, B1, C1
- High-Risk HPV Calibrator (HRC) replicates in microplate wells D1, E1, F1
- Low-Risk HPV Quality Control (QC1-LR) in microplate well G1
- High-Risk HPV Quality Control (QC2-HR) in microplate well H1

Position of calibrators, quality controls and samples on the microplate:



Once denatured, the calibrators and controls may be stored at 2–8°C overnight or at –20°C for up to 3 months. A maximum of 3 freeze/thaw cycles may be performed with a maximum of 2 hours at room temperature during each thaw cycle.

Follow this procedure if performing RCS-automated testing on the same or following day

1. Pipet the denatured calibrators and quality controls into the applicable positions on the hybridization microplate.
2. Cover the hybridization microplate with a new microplate lid.
3. Proceed with RCS-automated testing (refer to *Rapid Capture System User Manual – Performing digene HC2 DNA Tests Using QIAAsymphony SP Processed Samples* for additional instructions) or store overnight at 2–8°C.

Follow this procedure if performing RCS-automated testing after the following day

1. Store the denatured calibrators and quality controls at –20°C.
2. Cover the hybridization microplate containing only the denatured sample extracts with a new microplate lid and store for up to 3 days at 2–8°C.
3. On the day of testing, allow the denatured calibrators and quality controls and the microplate containing the sample extracts equilibrate to 20–25°C.

4. Remove the microplate lid and pipet the denatured calibrators and quality controls into the applicable positions on the microplate.
5. Cover the hybridization microplate with a new microplate lid.
6. Proceed with RCS-automated testing (refer to *Rapid Capture System User Manual – Performing digene HC2 DNA Tests Using QIAasymphony SP Processed Samples* for additional instructions).

Cleaning QIAasymphony SP

1. Remove the reagent cartridge(s) (RC). If the reagent cartridge (RC) is only partially used, seal it with the provided Reuse Seal Strips immediately after the protocol run to avoid evaporation.
Note: Refer to *QIAasymphony DSP HPV Media Kit Instructions for Use (Handbook)* for information about storage information.
2. Visually inspect the sample tubes. Exclude from further testing any sample that was not transferred during the protocol run.
3. Discard the used sample tubes and waste according to national and local safety regulations.
4. Clean QIAasymphony SP.
Follow the maintenance instructions in the user manuals supplied with your instrument.
5. Close the instrument drawers and power OFF QIAasymphony SP.

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