

April 2019

QIAsymphony[®] RGQ Application Sheet

artus[®] VZV QS-RGQ Kit (sample type: CSF)

R2

IVD

CE

REF

4502363

artus VZV QS-RGQ Kit, version 1



Check availability of new electronic labeling revisions at www.qiagen.com/products/artusvzvpcrkitce.aspx before test execution.

General Information

| | |
|---|--|
| Kit | <i>artus</i> VZV QS-RGQ Kit, Version 1 (cat.no. 4502363) |
| Validated sample material | CSF |
| Front-end purification | QIA Symphony DSP Virus/Pathogen Mini Kit (cat. no. 937036) |
| Sample volume (including excess volume) | 350 µl |
| Assay Parameter Set | <i>artus_VZV_CSF200_V5</i> |
| Default Assay Control Set | <i>Cellfree200_V7_DSP_artus_VZV</i> |
| Elution volume | 60 µl |
| Required software version | Version 4.0 or higher |
| Master mix volume | 30 µl |
| Template volume | 20 µl |
| Number of reactions | 6–24 |
| Runtime on AS module | For 6 reactions: approximately 9 minutes For 72 reactions: approximately 35 minutes |

Materials Required but Not Provided

Purification kit

- QIASymphony DSP Virus/Pathogen Mini Kit (cat. no. 937036)

Adapters for the QIASymphony SP

- Elution Microtube Rack QS (Cooling Adapter, EMT, v2, Qsym, cat. no. 9020730)
- Transfer frame
- Tube Insert 3B (Insert, 2.0ml v2, samplecarr. (24), Qsym, cat. no. 9242083)

Consumables for the QIASymphony SP

- Sample Prep Cartridges, 8-well (cat. no. 997002)
- 8-Rod Covers (cat. no. 997004)
- Filter-Tips, 1500 µl (cat. no. 997024)
- Filter-Tips, 200 µl (cat. no. 990332)
- Elution Microtubes CL (cat. no. 19588)
- Tip disposal bags (cat. no. 9013395)
- Micro tubes 2.0 ml Type H or Micro tubes 2.0 ml Type I (Sarstedt®, cat. nos. 72.693 and 72.694, www.sarstedt.com) for use with samples and internal controls

Adapters and reagent holders for the QIASymphony AS

- Reagent holder 1 QS (Cooling Adapter, Reagent Holder 1, Qsym, cat. no. 9018090)
- RG Strip Tubes 72 QS (Cooling Adapter, RG Strip Tubes 72, Qsym, cat. no. 9018092)

Consumables for the QIASymphony AS

- Strip Tubes and Caps, 0.1 ml (cat. no. 981103)
- Tubes, conical, 2 ml, Qsym AS (cat. no. 997102) or Micro tubes 2.0 ml Type I (Sarstedt, cat. no. 72.694.005)
- Alternatively: Tubes, conical, 5 ml, Qsym AS (cat. no. 997104) or Tubes with flat base from PP (Sarstedt, cat. no. 60.558.001)
- Filter-Tips, 1500 µl (cat. no. 997024)
- Filter-Tips, 200 µl (cat. no. 990332)
- Filter-Tips, 50 µl (cat. no. 997120)
- Tip disposal bags (cat. no. 9013395)

Specimen Handling and Storage

| | |
|--------------------|---|
| Sample collection | CSF |
| Sample storage | Samples should be stored at 2–8°C, or at –20°C |
| Sample transport | Shatterproof transport Shipment within 24 hours Mail shipment according to legal instructions for the transport of pathogen material* |
| Sample preparation | Prevent formation of foam in or on the samples Samples should be equilibrated to room temperature (15–25°C) before starting the run. |

* International Air Transport Association (IATA). Dangerous Goods Regulations.

Procedure

Preparation of carrier RNA and addition of the internal control to the samples

Using the QIAasymphony DSP Virus/Pathogen Mini Kit in combination with the *artus* VZV QS-RGQ Kit requires introduction of the internal control (VZV IC) into the purification procedure to monitor the efficiency of sample preparation and downstream assay.

Internal controls must be added with carrier RNA (CARRIER)–Buffer AVE (AVE) mixture, and the total volume of the internal control–carrier RNA (CARRIER)–Buffer AVE (AVE) mixture remains 120 µl.

The table represents the addition of internal control to the isolation at a ratio of 0.1 µl per 1 µl elution volume. We recommend preparing fresh mixtures for each run just before use.

Alternatively, the “IC Calculator” Tool in the QIAasymphony Management Console may be used.

| Component | Volume (µl) (Sarstedt tubes)* | Volume (µl) (Corning tubes)† |
|--|----------------------------------|---------------------------------|
| Stock carrier RNA (CARRIER) | 2.5 | 2.5 |
| Internal control‡ | 9 | 9 |
| Buffer AVE | 108.5 | 108.5 |
| Final volume per sample (excluding dead volume) | 120 | 120 |
| Total volume for n samples | $(n \times 120) + 360^{\S}$ | $(n \times 120) + 600^{\P}$ |

* Micro tubes 2.0 ml Type H and Micro tubes 2.0 ml Type I, Sarstedt cat. nos. 72.693 and 72.694.

† Tubes 14 ml, 17 x 100 mm polystyrene round-bottom (Corning® Inc., cat. no. 352051; Becton Dickinson was the previous supplier of this tube and Corning Inc. is now the new supplier).

‡ The calculation of the amount of internal control is based on the initial elution volumes (90 µl). Additional void volume depends on the type of sample tube used.

§ Internal control mixture corresponding to 3 additional samples (i.e., 360 µl) is required. Do not fill more than 1.92 ml total volume (corresponding to a maximum of 13 samples. These volumes are specific for Micro tubes 2.0 ml Type H and Micro tubes 2.0 ml Type I, Sarstedt cat. nos. 72.693 and 72.694).

¶ Internal control mixture corresponding to 5 additional samples (i.e., 600 µl) is required. Do not fill more than 13.92 ml total volume (corresponding to a maximum of 111 samples. These volumes are specific for Tubes 14 ml, 17 x 100 mm polystyrene round-bottom, Corning Inc., cat. no. 352051; Becton Dickinson was the previous supplier of this tube and Corning Inc. is now the new supplier).

QIAsymphony SP setup

“Waste” drawer

| | |
|----------------------------|---------------------------------------|
| Unit box holder 1–4 | Empty unit boxes |
| Waste bag holder | Waste bag |
| Liquid waste bottle holder | Empty and install liquid waste bottle |

“Eluate” drawer

| | |
|-----------------|---|
| Elution rack | Elution Microtubes CL in Elution Microtube Rack QS and Transfer frame Use slot 1, cooling position |
| Elution volume* | Preselected elution volume: 60 µl Initial elution volume: 90 µl |

* The elution volume is preselected for the protocol. This is the minimum accessible volume of eluate in the final elution tube. The initial volume of elution solution is required to ensure that the actual volume of eluate is the same as the preselected volume.

“Reagents and Consumables” drawer

| | |
|--------------------------------|---|
| RC Position 1 and 2 | Load 1 reagent cartridge (RC) for up to 96 samples |
| Tip rack holder positions 1–18 | Load sufficient racks of disposable filter-tips, 200 µl and 1500 µl (see “Required plasticware for 1–4 sample batches”, page 7) |
| Unit box holder positions 1–4 | Load unit boxes containing sample prep cartridges and 8-Rod Covers (see “Required plasticware for 1–4 sample batches”, page 7) |

“Sample” drawer

| | |
|---|--|
| Sample type | CSF |
| Sample volume (including excess volume) | 350 µl |
| Sample tubes | Micro tubes 2.0 ml Type H or Micro tubes 2.0 ml Type I (Sarstedt, cat. nos. 72.693 and 72.694) |
| Insert | Tube Insert 3B (cat. no. 9242083) |

Required plasticware for 1–4 sample batches

| Component | One batch, 24 samples* | Two batches, 48 samples* | Three batches, 72 samples* | Four batches, 96 samples* |
|--------------------------------------|---------------------------|-----------------------------|-------------------------------|------------------------------|
| Disposable filter-tips, 200 µl†‡ | 30 | 54 | 78 | 102 |
| Disposable filter-tips, 1500 µl†‡ | 101 | 182 | 271 | 354 |
| Sample prep cartridges§ | 21 | 42 | 63 | 84 |
| 8-Rod Covers¶ | 3 | 6 | 9 | 12 |

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

† 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.

QIAsymphony AS setup

Consumables

During the setup, the appropriate positions for each consumable on the QIAsymphony AS module are indicated on the touchscreen of the instrument.

| Consumable | Name on touchscreen | For use with adapter/ reagent holder |
|--|---|---|
| Strip Tubes and Caps, 0.1 ml (250) | QIA#981103 *StripTubes 0.1 | RG Strip Tubes 72 QS |
| Tubes, conical, 2 ml, Qsym AS (500) ^{†‡} | QIA#997102 *T2.0 ScrewSkirt [§] | Reagent holder 1 QS |
| Tubes, conical, 5 ml, Qsym AS (500) ^{†‡} | QIA#997104 *T5.0 ScrewSkirt [§] | Reagent holder 1 QS |

* Indicates labware that can be cooled using a cooling adapter with bar code.

[†] For master mix components, system-prepared master mix, assay standards, and assay controls.

[‡] Alternatively, the Sarstedt tubes described in "Material Required but Not Provided", page 3, can be used.

[§] The suffix "(m)" in the touchscreen indicates that liquid level calculations for the respective tube have been optimized for reagents forming a concave meniscus.

Adapters and reagent holders

| Rack/reagent holder | Name | Number required [¶] |
|---------------------|----------------------|------------------------------|
| Reagent holders | Reagent holder 1 QS | 1 |
| Sample racks | RG Strip Tubes 72 QS | 1 |

[¶] Calculated for an assay run with 72 reactions.

Filter-tips

Load tip racks starting with tip slots 1, 2 and 3 in the "Eluate and Reagents" drawer, and then load tip racks into tip slots 7, 8 and 9 in the "Assays" drawer.

| Consumable | Name on touchscreen | Minimum number for 24 reactions | Minimum number for 72 reactions |
|-----------------------------|------------------------|------------------------------------|------------------------------------|
| Filter-Tips, 1500 µl (1024) | 1500 µl | 4 | 6 |
| Filter-Tips, 200 µl (1024) | 200 µl | 10 | 9 |
| Filter-Tips, 50 µl (1024) | 50 µl | 25 | 73 |
| Tip Disposal Bags | – | 1 | 1 |

PCR on the Rotor-Gene Q*

Please refer to the software-specific protocol sheet *Settings to run artus QS-RGQ Kits* at www.qiagen.com/products/artusvzvpcrkite.aspx for protocol details.

Specific settings for the *artus* VZV QS-RGQ Kit

With Rotor-Gene® software 2.1 or higher, the specific settings are shown below.

| | |
|------------------------------|--|
| Reaction Volume (µl) | 50 |
| Hold | Hold Temperature: 95 degrees Hold Time: 10 minutes |
| Cycling | 45 times 95 degrees for 15 seconds 65 degrees for 30 seconds (Acquire on Green, Orange and activate touchdown function for 10 cycles) 72 degrees for 20 seconds |
| Auto-Gain Optimisation Setup | 65 degrees (Samples: Green; IC: Orange) |

Interpretation of results

This section describes interpretation of results on the Rotor-Gene Q. Review also the sample status information from the QIAAsymphony SP/AS result files for analysis of the complete sample-to-result workflow. Only samples with a valid status should be used.

The *artus* VZV QS-RGQ Kit can be run on the Rotor-Gene Q using manual analysis with Rotor-Gene Q software 2.1 or higher. The following sections describe interpretation of results using Rotor-Gene Q software 2.1 or higher.

* If applicable, Rotor-Gene Q 5plex HRM instrument with a production date of January 2010 or later. The production date can be obtained from the serial number on the back of the instrument. The serial number is in the format "mmyynnn" where "mm" indicates the production month in digits, "yy" indicates the last two digits of the production year, and "nnn" indicates the unique instrument identifier.

Signal detection and conclusions

| Signal in channel Cycling Green | Signal in channel Cycling Orange | Quantitative result (copies/ml) | Interpretation |
|------------------------------------|-------------------------------------|------------------------------------|--|
| Yes | Yes | <80.7 | Valid result: VZV DNA detected, <500 copies/ml. Quantitation not possible since the quantitative result is below limit of detection. Reproducibility of the positive result is not assured. |
| Yes | Yes | ≥80.7 and <500 | Valid result: VZV DNA detected, <500 copies/ml. Quantitation not possible since the quantitative result is below the linear range of the assay. |
| Yes | Yes/No* | ≥500 and ≤1 x 10 ⁸ | Valid result: VZV DNA detected at the calculated concentration. Quantitative result is within the linear range of the assay. |
| Yes | Yes/No* | >1 x 10 ⁸ | Valid result: VZV DNA detected, >1 x 10 ⁸ copies/ml. Quantitation not possible since the quantitative result is above the linear range of the assay.† |
| No | Yes | – | Valid result: No VZV DNA is detectable. ‡ |
| No | No | – | Invalid result: No result can be concluded. § |

* In this case, the detection of a signal in the Cycling Orange channel is dispensable, since high initial concentrations of VZV DNA (positive signal in the Cycling Green channel) can lead to a reduced or absent fluorescence signal of the internal control in the Cycling Orange channel (competition).

† If quantitation is desired, dilute the sample with VZV-free CSF and reprocess. Multiply the quantitative result from the reprocessed sample by the dilution factor.

‡ If the C_T value for the internal control of a negative sample is more than 3 cycles higher than the C_T value for the internal control of the no template control in the run (C_T IC Sample – C_T IC NTC >3), then the sample should be treated as invalid. No result can be concluded.

§ Information regarding error sources and their solution can be found in “Troubleshooting Guide” of the artus VZV QS-RGQ Kit Handbook.

Threshold setup for the PCR analysis

The optimal threshold settings for a given combination of Rotor-Gene Q instrument and *artus* QS-RGQ Kit should be set empirically by testing each individual combination since it is a relative value depending on the overall diagnostic workflow. The threshold can be set at a preliminary value of 0.04 for the analysis of the first PCR run, but this value should be fine-tuned in a comparative analysis of the next runs of the workflow. The threshold should be set manually just above the background signal of the negative controls and negative samples. The mean threshold value calculated from these experiments will most likely work for the majority of future runs, but the user should nevertheless review the generated threshold value at regular intervals. The threshold value will usually be in the range of 0.03–0.05 and should be rounded to no more than three decimal places.

Quantitation

The quantitation standards (VZV QS 1–4) in the *artus* VZV QS-RGQ Kit are treated as previously purified samples and the same volume is used (20 µl). To generate a standard curve on Rotor-Gene Q Instruments, all 4 quantitation standards should be used and defined in the **Edit Samples** dialog box on the Rotor-Gene Q instrument as standards with the specified concentrations (see the instrument user manual).

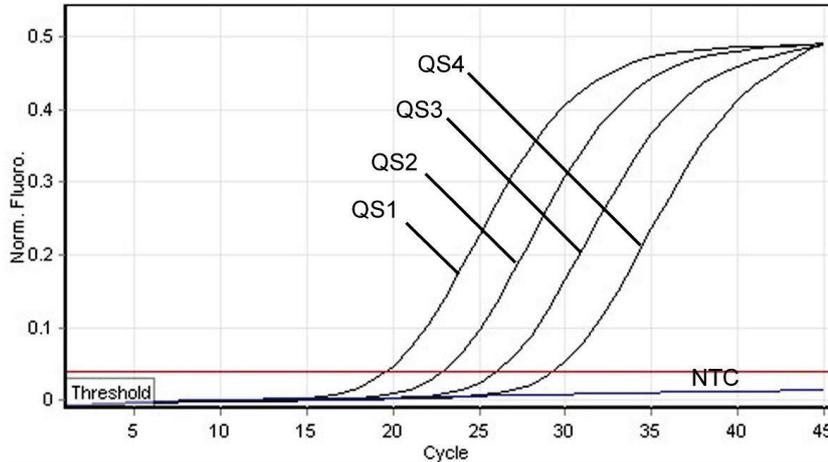
Note: The quantitation standards are defined as copies/µl in the eluate. The following equation has to be applied to convert the values determined using the standard curve into copies/ml of sample material.

$$\text{Result in sample material (copies/ml)} = \frac{\text{Result in eluate (copies/}\mu\text{l)} \times \text{Initial Elution Volume (90 }\mu\text{l)}^*}{\text{Sample Volume (ml)}}$$

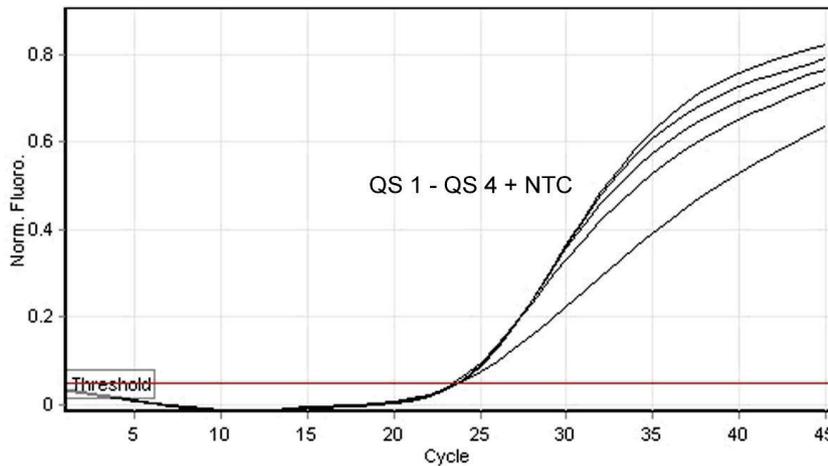
As a matter of principle, the initial sample volume should be entered in the equation above. This has to be considered when the sample volume has been changed prior to the nucleic acid extraction (e.g., reducing the volume by centrifugation or increasing the volume by adding to the volume required for the isolation).

* The calculation is based on the initial elution volumes (90 µl).

Examples of positive and negative PCR reactions



Detection of the quantitation standards (VZV QS 1–4) in fluorescence channel Cycling Green. NTC: No template control (negative control).



Detection of the internal control (IC) in fluorescence channel Cycling Orange with simultaneous amplification of the quantitation standards (VZV QS 1–4). NTC: No template control (negative control).

Document revision history

| | |
|----------------|--|
| R2, April 2019 | Removed footnote regarding setup of 216 assays. Changed to new versions of QIASymphony protocols. Updated required materials for setup of maximum 72 reactions. Added information on use of QMC "IC Calculator" tool. Updated naming of Corning labware (previously Becton Dickinson). Added specific run settings for Rotor-Gene Q (use of touchdown function, acquisitions). Added information to interpretation of results to include case "pathogen positive and IC negative". Removed instructions regarding use of Rotor-Gene AssayManager®; Changed wording from RT-PCR to PCR for clarification; Clarification of difference between eluate and sample concentration in quantitation calculation |
|----------------|--|

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