

# QIAasymphony<sup>®</sup> RGQ Application Sheet

---

## artus<sup>®</sup> HCV QS-RGQ Kit Application Sheet for plasma sample type

IVD



Check availability of new electronic labeling revisions at [www.qiagen.com/artus-HCV-QS-RGQ-el](http://www.qiagen.com/artus-HCV-QS-RGQ-el) before test execution. The current revision status is indicated by the issue date (format: month/year).

September 2015



---

Sample & Assay Technologies

---

## General information

<b>Kit</b>	<i>artus</i> HCV QS-RGQ Kit, Version 1, cat.no. 4518356
<b>Validated sample material</b>	Human EDTA plasma
<b>Front-end purification</b>	QIAasymphony DSP Virus/Pathogen Midi Kit (cat. no. 937055)
<b>Sample volume</b>	1200 $\mu$ l
<b>Assay Parameter Set</b>	<i>artus_HCV_plasma1000_V4</i> or higher
<b>Default Assay Control Set</b>	<i>Cellfree1000_V6_DSP_artus_HCV</i> or higher
<b>Elution volume</b>	60 $\mu$ l
<b>Required software version</b>	Version 4.0 or higher
<b>Master mix volume</b>	30 $\mu$ l
<b>Template volume</b>	20 $\mu$ l
<b>Number of reactions</b>	6–72
<b>Runtime on AS module</b>	Approximately 9 minutes for 6 reactions Approximately 35 minutes for 72 reactions

## Materials required but not provided

<b>Purification kit</b>	■ QIASymphony DSP Virus/Pathogen Midi Kit (cat. no. 937055)
<b>Adapters for the QIASymphony SP</b>	<ul style="list-style-type: none"> <li>■ Elution Microtube Rack QS (Cooling Adapter, EMT, v2, Qsym, cat. no. 9020730)</li> <li>■ Tube Insert 3B (Insert, 2.0ml v2, samplecarr. (24), Qsym, cat. no. 9242083)</li> </ul>
<b>Consumables for the QIASymphony SP</b>	<ul style="list-style-type: none"> <li>■ Sample Prep Cartridges, 8-well (cat. no. 997002)</li> <li>■ 8-Rod Covers (cat. no. 997004)</li> <li>■ Filter-Tips, 1500 <math>\mu</math>l (cat. no. 997024)</li> <li>■ Filter-Tips, 200 <math>\mu</math>l (cat. no. 990332)</li> <li>■ Elution Microtubes CL (cat. no. 19588)</li> <li>■ Tip disposal bags (cat. no. 9013395)</li> <li>■ Micro tubes 2.0 ml Type H or Micro tubes 2.0 ml Type I (Sarstedt®, cat. nos. 72.693 and 72.694, <a href="http://www.sarstedt.com">www.sarstedt.com</a>)</li> </ul>
<b>Adapters and reagent holders for the QIASymphony AS</b>	<ul style="list-style-type: none"> <li>■ Reagent holder 1 QS (Cooling Adapter, Reagent Holder 1, Qsym, cat. no. 9018090)</li> <li>■ Reagent holder 2 QS (Cooling Adapter, Reagent Holder 2, Qsym, cat. no. 9018089)</li> <li>■ RG Strip Tubes 72 QS (Cooling Adapter, RG Strip Tubes 72, Qsym, cat. no. 9018092)</li> </ul>
<b>Consumables for the QIASymphony AS</b>	<ul style="list-style-type: none"> <li>■ Strip Tubes and Caps, 0.1 ml (cat. no. 981103)</li> <li>■ Tubes, conical, 2 ml, Qsym AS (cat. no. 997102)* or Micro tubes 2.0 ml Type I (Sarstedt, cat. no. 72.694.005)</li> <li>■ Tube, conical, 5 ml, Qsym AS (cat. no. 997104)* or Tubes with flat base from PP (Sarstedt, cat. no. 60.558.001)</li> <li>■ Elution Microtubes CL (cat. no. 19588)</li> <li>■ Filter-Tips, 1500 <math>\mu</math>l (cat. no. 997024)</li> <li>■ Filter-Tips, 200 <math>\mu</math>l (cat. no. 990332)</li> <li>■ Filter-Tips, 50 <math>\mu</math>l (cat. no. 997120)</li> <li>■ Tip disposal bags (cat. no. 9013395)</li> </ul>

\* Please inquire for availability.

---

## Specimen handling and storage

<b>Sample collection</b>	Blood sample 5–10 ml EDTA blood 8x overhead inversion — no agitation! Heparinized human samples must not be used
<b>Sample storage</b>	Separation: 20 minutes centrifugation, 800–1600 x g within 24 hours post-collection Transfer the isolated plasma into a sterile polypropylene tube Virus encapsulated RNA stable at:*
	■ 4°C      days ■ -20°C    weeks ■ -70°C    months
<b>Sample transport</b>	Shatterproof transport Shipment within 24 hours Mail shipment according to legal instructions for the transport of pathogen material† Blood samples should be shipped cool (2–8°C)
<b>Interfering substances</b>	Heparin ( $\geq 10$ IU/ml) affects the PCR. Samples collected in tubes containing heparin as an anticoagulant or samples from heparinized patients must not be used. Elevated levels of albumin ( $\leq 6$ g/dl), bilirubin ( $\leq 30$ mg/dl), lipids ( $\leq 1$ g/dl triglyceride), and hemolytic samples ( $\leq 2$ g/dl hemoglobin) do not influence the system.

\* Arbeitskreis Blut, V17 (09.1997), Bundesgesundheitsblatt 11/1997, p. 452–456.

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

---

## Procedure

### Preparation of Carrier RNA (CARRIER) and addition of the internal control to the samples

Using the QIAasymphony DSP Virus/Pathogen Midi Kit in combination with the *artus* HCV QS-RGQ Kit requires introduction of the internal control (Hep. C Virus RG 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  $\mu$ l.

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

<b>Component</b>	<b>Volume (<math>\mu</math>l) (Sarstedt® tubes)*</b>	<b>Volume (<math>\mu</math>l) (Corning® tubes)†</b>
Stock Carrier RNA (CARRIER)	5	5
Internal control‡	9	9
Buffer AVE	106	106
<b>Final volume per sample (excluding dead volume)</b>	<b>120</b>	<b>120</b>
<b>Total volume for n samples</b>	<b>(n x 120) + 360§</b>	<b>(n x 120) + 600¶</b>

\* 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®, cat. no. 352051).

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

§ Internal control mixture corresponding to 3 additional samples (i.e., 360  $\mu$ l) 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  $\mu$ l) 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, cat. no. 352051.

---

## QIAasymphony 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	Use slot 1, cooling position
Elution volume*	Preselected elution volume: 60 $\mu$ l Initial elution volume: 90 $\mu$ l

\* 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 48 samples or 2 new Reagent Cartridges (RC) for up to 96 samples
Tip rack holder position 1–4	Load sufficient racks of disposable Filter-Tips, 200 $\mu$ l (see “Required plasticware for 1–4 sample batches”, page 7)
Tip rack holder position 5–18	Load sufficient racks of disposable Filter-Tips, 1500 $\mu$ l (see “Required plasticware for 1–4 sample batches”, page 7)
Unit box holder position 1–3	Load 3 unit boxes containing Sample Prep Cartridges
Unit box holder position 4	Load 1 unit box containing 8-Rod Covers

## “Sample” drawer

<b>Sample type</b>	Plasma
<b>Sample volume (including excess volume)</b>	1200 $\mu$ l
<b>Sample tubes</b>	Micro tubes 2.0 ml Type H or Micro tubes 2.0 ml Type I (Sarstedt, cat. nos. 72.693 and 72.694)
<b>Insert</b>	Tube Insert 3B (cat. no. 9242083)

## Required plasticware for 1–4 sample batches

	<b>1 batch, 24 samples*</b>	<b>2 batches, 48 samples*</b>	<b>3 batches, 72 samples*</b>	<b>4 batches, 96 samples*</b>
Disposable Filter-Tips, 200 $\mu$ l <sup>†‡</sup>	28	52	80	104
Disposable Filter-Tips, 1500 $\mu$ l <sup>†‡</sup>	85	162	247	324
Sample Prep Cartridges <sup>§</sup>	21	42	63	84
8-Rod Covers <sup>¶</sup>	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 one inventory scan per Reagent Cartridge (RC).

§ There are 28 sample prep cartridges/unit box.

¶ There are twelve 8-Rod Covers/unit box.

# QIAasymphony AS setup

## Consumables

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

Consumables	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) <sup>†‡</sup>	QIA#997102 *T2.0 ScrewSkirt <sup>§</sup>	Reagent holder 1 QS Reagent holder 2 QS
Tube, conical, 5 ml, Qsym AS (500) <sup>†‡</sup>	QIA#997104 *T5.0 ScrewSkirt <sup>§</sup>	Reagent holder 1 QS Reagent holder 2 QS
Elution Microtubes CL (24 x 96)	QIA#19588 * EMTR	Elution Microtube Rack QS

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

<sup>†</sup> For master mix components, system-prepared master mix, assay standards, and assay controls.

<sup>‡</sup> Alternatively, the Sarstedt tubes described in “Materials required but not provided”, page 2, can be used.

<sup>§</sup> 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 <sup>¶</sup>
Sample rack	Elution Microtube Rack QS	1
Reagent holders	Reagent holder 1 QS	1
Assay racks	RG Strip Tubes 72 QS	1

<sup>¶</sup> 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.

<b>Consumable</b>	<b>Name on touchscreen</b>	<b>Minimum number for 24 reactions</b>	<b>Minimum number for 72 reactions</b>
Filter-Tips, 1500 $\mu$ l (1024)	1500 $\mu$ l	5	6
Filter-Tips, 200 $\mu$ l (1024)	200 $\mu$ l	10	10
Filter-Tips, 50 $\mu$ l (1024)	50 $\mu$ l	25	73
Tip Disposal Bags	–	1	1

---

## RT-PCR on the Rotor-Gene Q

The *artus* HCV QS-RGQ Kit must be run on Rotor-Gene Q using manual analysis with Rotor-Gene Q Software 2.3 or higher. Set the following parameters for the run.

Setting	Parameter
Reaction volume ( $\mu$ L)	50
Hold	Temperature: 50 degrees Time: 30 minutes
Hold 2	Temperature: 95 degrees Time: 15 minutes
Cycling	50 time(s) 95 degrees for 30 seconds 50 degrees for 60 seconds 72 degrees for 30 seconds
Auto-Gain Optimisation Setup	50 degrees (Samples: Green; IC: Orange)

For more detailed instructions, refer to "Settings to run *artus* QS-RGQ Kits (Rotor-Gene Q Software 2.3, or higher)" at [www.qiagen.com/artus-HBV-QS-RGQ-eL](http://www.qiagen.com/artus-HBV-QS-RGQ-eL).

### Interpretation of results

This section describes interpretation of results on Rotor-Gene Q using Rotor-Gene Q Software 2.3 or higher. Review also the sample status information from the QIASymphony SP/AS result files for analysis of the complete sample-to-result workflow. Only samples with a valid status should be used.

Information regarding error sources and their solution can be found in "Troubleshooting guide" of the *artus* HCV QS-RGQ Kit Handbook.

### Analysis settings for PCR

To ensure data generated will be analogous to the performance characteristics of the *artus* HCV QS-RGQ Kit, use the following parameters to analyze all data generated using the *artus* HCV QS-RGQ Kit.

---

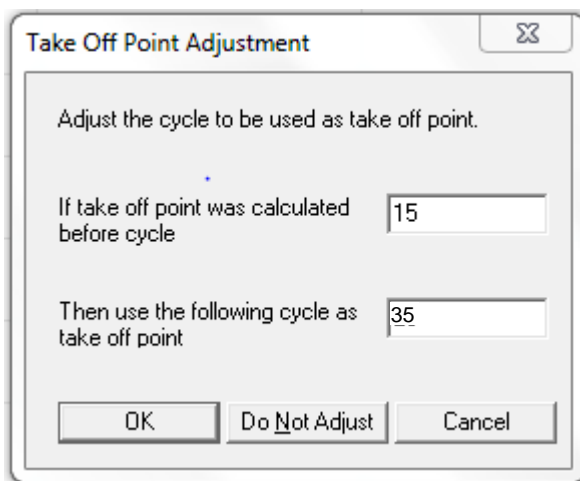
### Analysis settings for PCR

Channel	Target	Threshold	Dynamic tube	Slope correct	Take-Off Adjustment*
Green	HCV	0.05	On	Off	15/35
Orange	IC <sup>†</sup>	0.03	On	On	15/35

\* Take-Off Adjustment requires RG software version 2.3 or higher.

† IC: Internal Control.

1. After the run is finished, analyze the data using the Rotor-Gene Q Software.
2. Open the run file (if closed) and select "Analysis" and "Cycling A. Green" for the analysis of HCV viral load.
3. Select "Dynamic tube".
4. Make sure "Slope correct" is not selected.
5. Select "Take Off Adj." and enter "15" in the top and "35" in the bottom cell.



6. Set the graph to linear scale and set the threshold to "0.05".  
Data can be exported by right-clicking in the "Results" window and using the "Export to Excel" function.
7. For the analysis of the IC values, select "Analysis" and "Cycling A. Orange".
8. Select "Dynamic tube".
9. Select "Slope correct".

- 
10. Select "Take Off Adj." and enter "15" in the top and "35" in the bottom cell.

Take Off Point Adjustment

Adjust the cycle to be used as take off point.

If take off point was calculated before cycle

Then use the following cycle as take off point

OK Do Not Adjust Cancel

11. Set the graph to linear scale and set the threshold to "0.03".

Data can be exported by right-clicking in the "Results" window and using the "Export to Excel" function.

12. Convert titer values from IU/ $\mu$ l to IU/ml using the equation provided in "Quantitation", page 13.

---

## Quantitation

The quantitation standards (Hep. C Virus RG QS 1–4) in the *artus* HCV 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, all 4 quantitation standards should be used and defined in the “Edit Samples” dialog box on Rotor-Gene Q as standards with the specified concentrations. Refer to the applicable user manual for additional instructions.

**Note:** The quantitation standards are defined as IU/µl and have been calibrated using the International HCV standard (WHO). The following equation has to be applied to convert the values determined using the standard curve into IU/ml of sample material. The calculation is based on the initial elution volumes (90 µl).

$$\text{Result (IU/ml)} = \frac{\text{Result (IU/}\mu\text{l)} \times 90 \mu\text{l (initial elution volume)}^*}{\text{Sample volume (ml)}}$$

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).

## Conversion factor

One IU/ml corresponds to 1.21 copies/ml for detection of HCV RNA on the Rotor-Gene Q. The conversion factor was established by a regression analysis of multiple dilution series compared against a reference method reporting in copies/ml.

---

\* The standard has been calibrated using the International HCV standard (WHO).

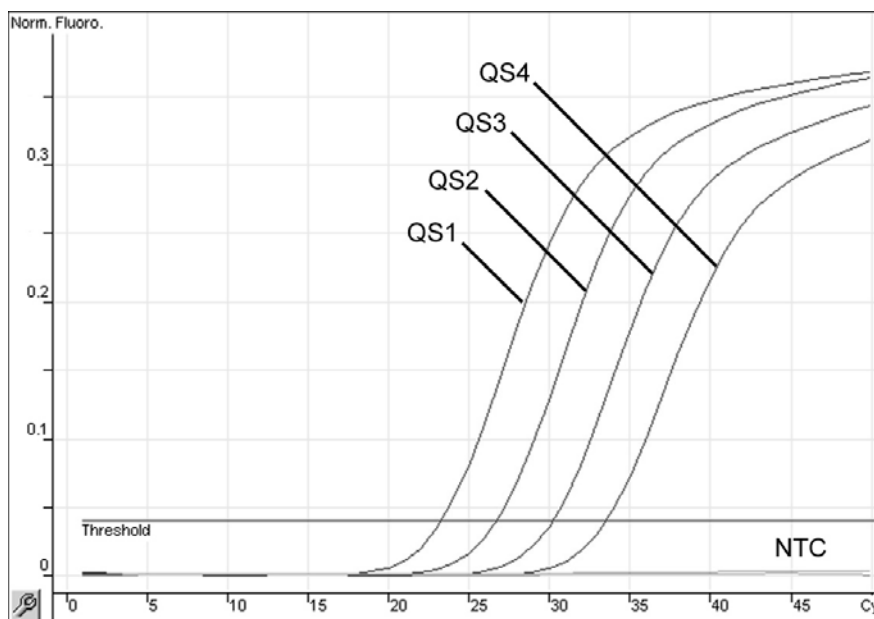
## Signal detection and conclusions

Signal in channel Cycling Green	Signal in channel Cycling Orange	Quantitative result (IU/ml)	Interpretation
Yes	Yes	<21	Valid result: HCV RNA detected, <35 IU/ml* Quantitation not possible since the quantitative result is below limit of detection. Reproducibility of the positive result is not assured.
Yes	Yes	≥21 and <35	Valid result: HCV RNA detected, <35 IU/ml* Quantitation not possible since the quantitative result is below the linear range of the assay.
Yes	Yes/No <sup>†</sup>	≥35 and ≤1.77 x 10 <sup>7</sup>	Valid result: HCV RNA detected at the calculated concentration Quantitative result is within the linear range of the assay.
Yes	Yes/No <sup>†</sup>	>1.77 x 10 <sup>7</sup>	Valid result: HCV RNA detected, >1.77 x 10 <sup>7</sup> IU/ml Quantitation not possible since the quantitative result is above the linear range of the assay. <sup>†</sup>
No	Yes	–	Valid result: No HCV RNA is detectable.
Yes	No	<35	Invalid result: No result can be concluded.
No	No	–	Invalid result: No result can be concluded.

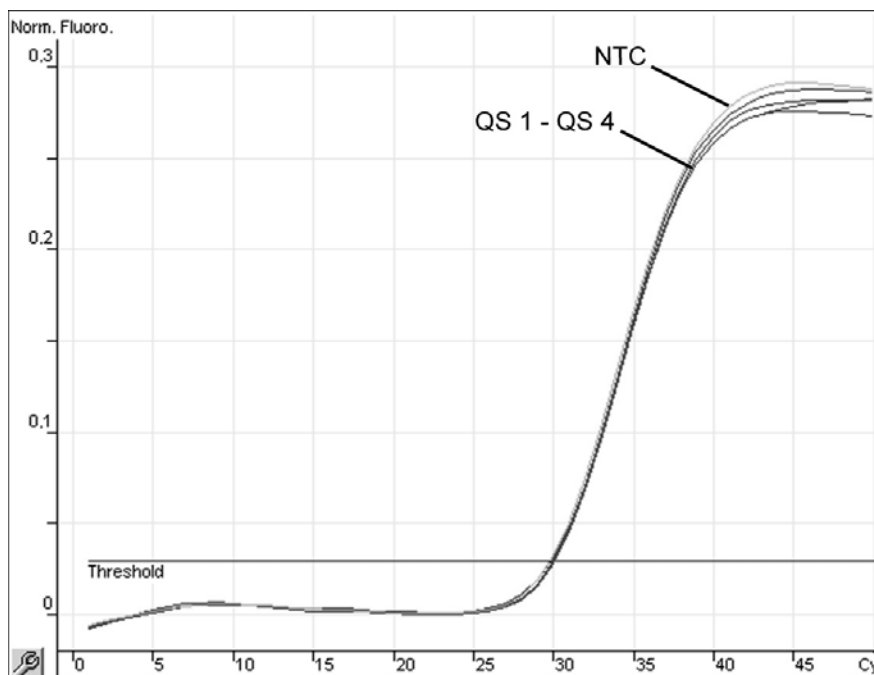
\* If the  $C_T$  value for the internal control of a sample below the linear range or of a negative sample is more than 3 cycles higher than the  $C_T$  value for the internal control of the no template control in the run ( $C_{T\ IC\ Sample} - C_{T\ IC\ NTC} > 3$ ), then the sample should be treated as invalid. No result can be concluded.

<sup>†</sup> In this case, the detection of a signal in the Cycling Orange channel is dispensable, since high initial concentrations of HCV RNA (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).

## Examples of positive and negative PCR reactions



**Detection of the quantitation standards (Hep. C Virus 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 (Hep. C Virus QS 1–4).** NTC: No template control (negative control).

---

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](http://www.qiagen.com) or can be requested from QIAGEN Technical Services or your local distributor.

[www.qiagen.com](http://www.qiagen.com)

Brazil ☎ 0800-557779

Trademarks: QIAGEN®, QIASymphony®, *artus*®, Rotor-Gene® (QIAGEN Group); Corning® (Corning Incorporated); Sarstedt® (Sarstedt AG and Co.).

Sep-15 HB-2002-S01-001 © 2015 QIAGEN, all rights reserved.



---

Sample & Assay Technologies