

# QIASymphony® RGQ Application Sheet

## QIASymphony RGQ application artus® HI Virus-1 QS-RGQ Kit (sample type: plasma)



Check availability of new electronic labeling revisions at [www.qiagen.com/products/artushivirusrtpcrkitce.aspx](http://www.qiagen.com/products/artushivirusrtpcrkitce.aspx) before test execution. The current revision status is indicated by the issue date (format: month/year).

### General information

Kit	artus HI Virus-1 QS-RGQ Kit, Version 1, <b>REF</b> 4513363, 4513366
Validated sample material	Human EDTA plasma
Front-end purification	QIASymphony DSP Virus/Pathogen Midi Kit (cat. no. 937055)
Sample volume (including excess volume)	1200 µl
Assay Parameter Set	artus_HIV plasma1000_V4
Default Assay Control Set	Cellfree1000_V6_DSP artus HIV
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 or 6–72*
Runtime on AS module	For 6 reactions: approximately 9 minutes For 72 reactions: approximately 35 minutes

\* When running multiple assay runs, ensure that the limit of 72 reactions and 1 assay rack adapter is not exceeded. Avoid extended incubation time (>30 minutes) between completion of the assay run and transfer to the Rotor-Gene® Q.

May 2012



Sample & Assay Technologies

## Materials required but not provided

Purification kit	■	QIASymphony DSP Virus/Pathogen Midi Kit (cat. no. 937055)
Adapters for the QIASymphony SP	■	Elution Microtube Rack QS (Cooling Adapter, EMT, v2, Qsym, cat. no. 9020730)
	■	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 $\mu$ l (cat. no. 997024)
	■	Filter-Tips, 200 $\mu$ l (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, <a href="http://www.sarstedt.com">www.sarstedt.com</a> ) 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)
	■	Reagent holder 2 QS (Cooling Adapter, Reagent Holder 2, Qsym, cat. no. 9018089)
	■	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)
	■	Tube, conical, 5 ml, Qsym AS (cat. no. 997104)* or Tubes with flat base from PP (Sarstedt, cat. no. 60.558.001)
	■	Reagent Bottles, 30 ml, Qsym AS (cat. no. 997108)
	■	Elution Microtubes CL (cat. no. 19588)
	■	Filter-Tips, 1500 $\mu$ l (cat. no. 997024)
	■	Filter-Tips, 200 $\mu$ l (cat. no. 990332)
	■	Filter-Tips, 50 $\mu$ l (cat. no. 997120)
	■	Tip disposal bags (cat. no. 9013395)

\* Please inquire for availability.

---

## Specimen handling and storage

Sample collection	Blood sample 5–10 ml EDTA blood 8x overhead mix — no agitation! Heparinized human samples must not be used
Sample storage	Separation: 20 minutes centrifugation, 800–1600 x g within 24 hours post-collection Transfer of the isolated plasma into a sterile polypropylene tube Virus encapsulated RNA stable at: * 4°C    days –20°C    weeks –70°C    months
Sample transport	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 to 8°C)
Interfering substances	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 and addition of the internal control to the samples

Using the QIA Symphony DSP Virus/Pathogen Midi Kit in combination with the *artus* HI Virus-1 QS-RGQ Kit requires introduction of the internal control (HI Virus-1 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.

Component	Volume ( $\mu$ l) (Sarstedt® tubes)*	Volume ( $\mu$ l) (BD™ tubes)†
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 (Becton Dickinson, 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, Becton Dickinson, cat. no. 352051).

---

## 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	We recommend using 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 6)
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 6)
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>One batch, 24 samples<sup>†</sup></b>	<b>Two batches, 48 samples<sup>†</sup></b>	<b>Three batches, 72 samples<sup>†</sup></b>	<b>Four batches, 96 samples<sup>†</sup></b>
<b>Disposable filter-tips, 200 <math>\mu</math>l<sup>‡§</sup></b>	28	52	76	100
<b>Disposable filter-tips, 1500 <math>\mu</math>l<sup>‡§</sup></b>	113	206	309	402
<b>Sample prep cartridges<sup>¶</sup></b>	21	42	54	72
<b>8-Rod Covers<sup>**</sup></b>	3	6	9	12

<sup>†</sup> Use of more than one internal control tube per batch and performing more than one inventory scan requires additional disposable filter tips.

<sup>‡</sup> There are 32 filter-tips/tip rack.

<sup>§</sup> Number of required filter-tips includes filter-tips for 1 inventory scan per reagent cartridge.

<sup>¶</sup> There are 28 sample prep cartridges/unit box.

<sup>\*\*</sup> 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.

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)*†	QIA#997102 *T2.0 ScrewSkirt‡	Reagent holder 1 QS Reagent holder 2 QS
Tube, conical, 5 ml, Qsym AS (500)*†	QIA#997104 *T5.0 ScrewSkirt‡	Reagent holder 1 QS Reagent holder 2 QS
Reagent Bottles, 30ml, Qsym AS (50)*	QIA#997108 *Bottle 30ml‡	Reagent holder 2 QS
Elution Microtubes CL (24 x 96)	QIA#19588 * EMTR	Elution Microtube Rack QS

\* For master mix components, system-prepared master mix, assay standards, and assay controls.

† Alternatively, the Sarstedt tubes described in “Materials required but not provided”, page 2, 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 <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.

Consumable	Name on touchscreen	Minimum number for 24 reactions	Minimum number for 72 reactions
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

Please refer to the software-specific protocol sheet “Settings to run *artus* QS-RGQ Kits” at [www.qiagen.com/products/artushivirusrf-pcrkitce.aspx](http://www.qiagen.com/products/artushivirusrf-pcrkitce.aspx).

### Specific settings for the *artus* HI Virus-1 QS-RGQ Kit

Rotor-Gene AssayManager automatically sets the parameters for the *artus* HI Virus-1 QS-RGQ Kit.

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

<b>Reaction Volume (<math>\mu</math>L)</b>	50
<b>Hold</b>	Hold Temperature: 50 deg Hold Time: 30 mins
<b>Hold 2</b>	Hold Temperature: 95 deg Hold Time: 15 mins
<b>Cycling</b>	50 time(s) 95 deg for 30 secs 50 deg for 60 secs 72 deg for 30 secs
<b>Auto-Gain Optimisation Setup</b>	50 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

Rotor-Gene AssayManager automatically interprets the PCR results and gives a conclusion and quantitative result.

The following sections describe interpretation of results using Rotor-Gene software 2.1 or higher.

### Signal detection and conclusions

Signal in channel Cycling Green	Signal in channel Cycling Orange	Quantitative result (IU/ml)	Interpretation
Yes	Yes	<76.4	Valid result: HIV-1 RNA detected, <100 IU/ml Quantitation not possible since the quantitative result is below limit of detection. Reproducibility of the positive result is not assured.
Yes	Yes	≥76.4 and <100	Valid result: HIV-1 RNA detected, <100 IU/ml Quantitation not possible since the quantitative result is below the linear range of the assay.
Yes	Yes	≥100 and ≤1.00 x 10 <sup>8</sup>	Valid result: HIV-1 RNA detected at the calculated concentration Quantitative result is within the linear range of the assay.
Yes	Yes	>1.00 x 10 <sup>8</sup>	Valid result: HIV-1 RNA detected, >1.00 x 10 <sup>8</sup> Quantitation not possible since the quantitative result is above the linear range of the assay.*
No	Yes	–	Valid result: No HIV-1 RNA is detectable.†
No	No	–	Invalid result: No result can be concluded.‡

\* If quantitation is desired, dilute the sample with HIV-free plasma and reprocess. Multiply the quantitative result from the reprocessed sample by the dilution factor.

† If the C<sub>T</sub> value for the internal control of a negative sample is more than 3 cycles higher than the C<sub>T</sub> value for the internal control of the no template control in the run (C<sub>T IC Sample</sub> – C<sub>T IC NTC</sub> >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 HI Virus-1 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 (HI Virus-1 RG QS 1–4) in the *artus* HI Virus-1 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 IU/µl.\* The following equation has to be applied to convert the values determined using the standard curve into IU/ml of sample material

$$\text{Result (IU/ml)} = \frac{\text{Result (IU/}\mu\text{l)} \times \text{Initial Elution Volume (90 }\mu\text{l)}^\dagger}{\text{Sample Volume (ml)}}$$

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).

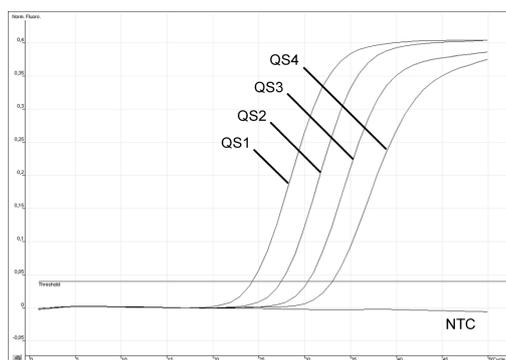
## Conversion factor

1 IU/ml corresponds to 0.45 copies/ml for detection of HIV-1 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.

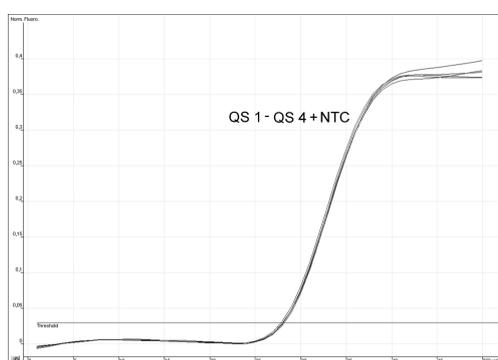
\* The standard has been calibrated using the International HIV standard (WHO).

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

## Examples of positive and negative PCR reactions



Detection of the quantitation standards (HI Virus-1 RG 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 (HI Virus-1 RG 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.

Trademarks: QIAGEN®, QIASymphony®, artus®, Rotor-Gene® (QIAGEN Group); BD™ (Becton, Dickinson and Company); Sarstedt® (Sarstedt AG and Co.).

© 2012 QIAGEN, all rights reserved.

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

Canada = 800-572-9613

Ireland = 1800 555 049

Norway = 800-18859

China = 021-3865-3865

Italy = 800-787980

Singapore = 65-67775366

Denmark = 80-885945

Japan = 03-6890-7300

Spain = 91-630-7050

Australia = 1-800-243-800

Finland = 0800-914416

Korea (South) = 1544 7145

Sweden = 020-790282

Austria = 0800/281010

France = 01-60-920-930

Luxembourg = 8002 2076

Switzerland = 055-254-22-11

Belgium = 0800-79612

Germany = 02103-29-12000

Mexico = 01-800-7742-639

UK = 01293-422-911

Brazil = 0800-557779

Hong Kong = 800 933 965

The Netherlands = 0800 0229592

USA = 800-426-8157



Sample & Assay Technologies