QIAsymphony® RGQ Application Sheet

artus® HI Virus-1 QS-RGQ Kit
Application Sheet for plasma sample type

IVD 

Check availability of new electronic labeling revisions at www.qiagen.com/artus-HIV1-QS-RGQ-eL before test execution. The current revision status is indicated by the issue date (format: month/year).

September 2015

QIAGEN®

Sample & Assay Technologies

General information

Kit	*artus* HI Virus-1 QS-RGQ Kit, Version 1, cat. no. 4513356
Validated sample material	Human EDTA plasma
Front-end purification	QIAsymphony DSP Virus/Pathogen Midi Kit (cat. no. 937055)
Sample volume	1200 µl
Assay Parameter Set	artus_HIV plasma1000_V4 or higher
Default Assay Control Set	Cellfree1000_V6_DSP_artus_HIV or higher
Elution volume	60 µl
Required software version	Version 4.0. or higher
Master mix volume	30 µl
Template volume	20 µl
Number of reactions	6–72
Runtime on AS module	Approximately 9 minutes for 6 reactions Approximately 35 minutes for 72 reactions

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 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)
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)
	■	Elution Microtubes CL (cat. no. 19588)
	■	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)

* Please inquire for availability.

Specimen handling and storage

Sample collection	Blood sample 5–10 ml EDTA blood 8x mix by inversion Heparinized human samples must not be used
Sample storage	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
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 (2–8°C)
Interfering substances	Heparin (≥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 (≤6 g/dl), bilirubin (≤30 mg/dl), lipids (≤1 g/dl triglyceride), and hemolytic samples (≤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 QIAsymphony 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 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.

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

* 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 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, 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	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 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 µl (see "Required plasticware for 1–4 sample batches", page 7)
Tip rack holder position 5–18	Load sufficient racks of disposable Filter-Tips, 1500 µl (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

Sample type	Plasma
Sample volume (including excess volume)	1200 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

	1 batch, 24 samples*	**3 batches, 48 samples***	**3 batches, 72 samples***	**4 batches, 96 samples***
Disposable Filter-Tips, 200 µl†‡	28	52	80	104
Disposable Filter-Tips, 1500 µl†‡	85	162	247	324
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 one inventory scan per Reagent Cartridge (RC).

§ 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 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
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§
Sample rack	Elution Microtube Rack QS	1
Reagent holders	Reagent holder 1 QS	1
Assay 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	5	6
Filter-Tips, 200 µl (1024)	200 µl	10	10
Filter-Tips, 50 µl (1024)	50 µl	25	73
Tip Disposal Bags	–	1	1

RT-PCR on the Rotor-Gene® Q

The *artus* HI Virus-1 QS-RGQ Kit must be run on Rotor-Gene Q using manual analysis with Rotor-Gene Q 2.3 or higher. Set the following parameters for the run.

Setting	Parameter
Reaction volume (µL)	50
Hold	Temperature: 50 degrees Time: 30 minutes
Hold 2	Temperature: 95 degrees Time: 15 minutes
Cycling	50 time 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-HIV1-QS-RGQ-eL.

Interpretation of Results

This section describes interpretation of results on the 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.

Threshold setup for PCR analysis

To ensure data generated will be analogous to the performance characteristics of the *artus* HI Virus-1 QS-RGQ Kit, use the following parameters to analyze all data generated using the *artus* HI Virus-1 QS-RGQ Kit.

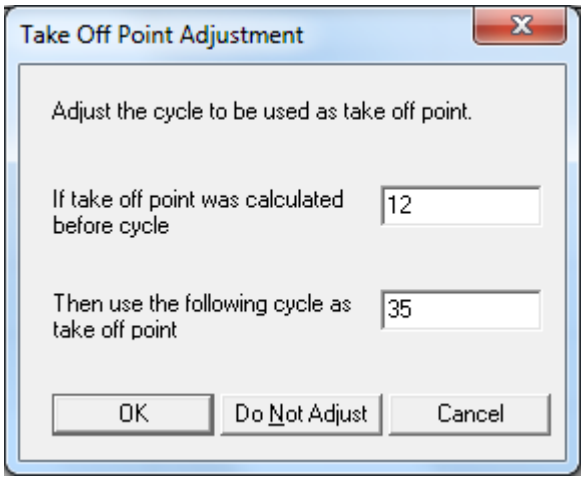
Analysis settings for PCR

Channel	Target	Dynamic tube	Threshold	Slope correct	Take-Off Adjustment*
Green	HIV	On	0.05	Off	12/35
Orange	IC†	On	0.03	On	15/35

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

† IC: Internal Control.

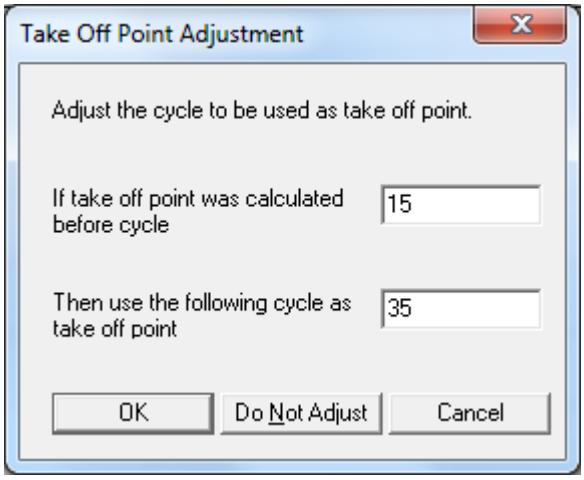
1. **After the run is finished, analyze the data using the Rotor-Gene Q Software.**
2. **Open the run file (if closed) and select "Analysis" and "Cycling A. Green" for the analysis of HIV titers.**
3. **Select "Dynamic tube".**
4. **Make sure "Slope correct" is not selected.**
5. **Select "Take Off Adj." and enter "12" 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.**



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/µl to IU/ml using the equation provided in "Quantitation," page 11.**

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, 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. 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 HIV 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)

$$\text{Result (IU/ml)} = \frac{\text{Result (IU/µl) x 90 µl (initial elution volume)}^{\dagger}}{\text{Sample volume (ml)}}$$

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

One 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.

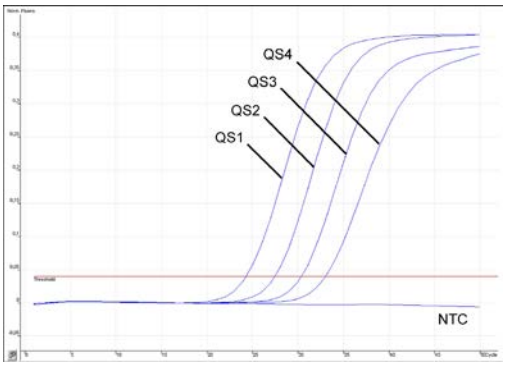
Signal detection and conclusions

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/No†	≥100 and ≤1.00 x 10^8	Valid result: HIV-1 RNA detected at the calculated concentration Quantitative result is within the linear range of the assay.
Yes	Yes/No†	>1.00 x 10^8	Valid result: HIV-1 RNA detected, >1.00 x 10^8 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.†
Yes	No	<100	Invalid result: No result can be concluded.
No	No	–	Invalid result: No result can be concluded.

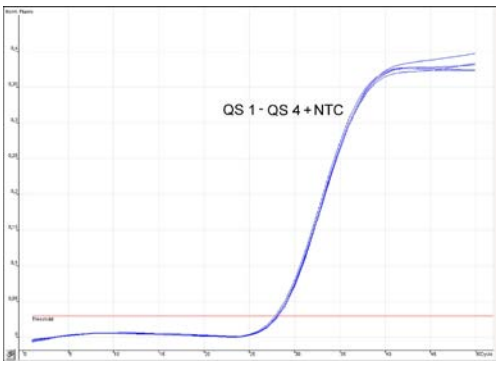
* If the C_T value for the internal control of a sample below the linear range or of a negative sample is more than 3 cycles higher than the C_T value for the internal control of the no template control in the run ($C_{T\ IC\ Sample} - C_{T\ IC\ NTC} > 3$), then the sample should be treated as invalid.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

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

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).

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