

QIAasymphony® RGQ Application Sheet

QIAasymphony RGQ application artus® HBV QS-RGQ Kit (sample type: plasma)



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General information

Kit	artus HBV QS-RGQ Kit, Version 1, REF 4506363, 4506366
Validated sample material	Human EDTA plasma
Front-end purification	QIAasymphony DSP Virus/Pathogen Midi Kit (cat. no. 937055)
Sample volume (including excess volume)	1200 µl
Assay Parameter Set	artus_HBV_plasma1000_V4
Default Assay Control Set	Cellfree1000_V6_DSP_artus_HBV
Elution volume	60 µl
Required software version	Version 4.0 or higher
Master mix volume	30 µl
Template volume	20 µl
Number of reactions	7–24 or 7–72*
Runtime on AS module	For 7 reactions: approximately 7 minutes For 72 reactions: approximately 35 minutes

* For assay setup for HBV, up to 216 (3 x 72) assays can be set up in one run on the QIAasymphony AS

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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 μ l (cat. no. 997024)
	■	Filter-Tips, 200 μ 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, 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)
	■	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 μ l (cat. no. 997024)
	■	Filter-Tips, 200 μ l (cat. no. 990332)
	■	Filter-Tips, 50 μ 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 the isolated plasma into a sterile polypropylene tube The sensitivity of the assay can be reduced if samples are frozen as a matter of routine or stored for a longer period of time. Virus encapsulated DNA 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 (≥ 10 IU/ml) affects the PCR. Samples collected in tubes containing heparin as an anticoagulant or samples from heparinized patients must not be used.
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.

* 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 QIAasymphony DSP Virus/Pathogen Midi Kit in combination with the *artus* HBV QS-RGQ Kit requires introduction of the internal control (HBV RG/TM 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 μ l.

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

Component	Volume (μ l) (Sarstedt® tubes)*	Volume (μ l) (BD™ 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 (Becton Dickinson, cat. no. 352051).

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

§ Internal control mixture corresponding to 3 additional samples (i.e., 360 μ 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 μ 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).

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 μ l Initial elution volume: 90 μ 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 positions 1–18	Load sufficient racks of disposable filter-tips, 200 μ l and 1500 μ l (see Required plasticware for 1–4 sample batches”, page 6)
Unit box holder position 1–4	Load unit boxes containing sample prep cartridges and 8-Rod Covers (see Required plasticware for 1–4 sample batches”, page 6)

“Sample” drawer

Sample type	Plasma
Sample volume (including excess volume)	1200 μ l
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

	One batch, 24 samples*	Two batches, 48 samples*	Three batches, 72 samples*	Four batches, 96 samples*
Disposable filter-tips, 200 μl^{†‡}	28	52	76	100
Disposable filter-tips, 1500 μl^{†‡}	113	206	309	402
Sample prep cartridges[§]	21	42	54	72
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.

QIA Symphony AS setup

Consumables

During the setup, the appropriate positions for each consumable on the QIA Symphony 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 [§]
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 μ l (1024)	1500 μ l	4	5
Filter-Tips, 200 μ l (1024)	200 μ l	9	8
Filter-Tips, 50 μ l (1024)	50 μ 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/artushbvpcrkitce.aspx.

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

Rotor-Gene AssayManager automatically sets the parameters for the *artus* HBV QS-RGQ Kit.

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

Reaction Volume (μL)	50
Hold	Hold Temperature: 95 deg Hold Time: 10 mins
Cycling	45 times 95 deg for 15 secs 55 deg for 30 secs 72 deg for 15 secs
Auto-Gain Optimisation Setup	55 degrees (Samples: Green; IC: Yellow)

Interpretation of results

This section describes interpretation of results on the Rotor-Gene Q. 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.

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 Yellow	Quantitative result (IU/ml)	Interpretation
Yes	Yes	<10.22	Valid result: HBV DNA detected, <31.6 IU/ml Quantitation not possible since the quantitative result is below limit of detection. Reproducibility of the positive result is not assured.
Yes	Yes	≥ 10.22 and <31.6	Valid result: HBV DNA detected, <31.6 IU/ml Quantitation not possible since the quantitative result is below the linear range of the assay.
Yes	Yes	≥ 31.6 and $\leq 2 \times 10^7$	Valid result: HBV DNA detected at the calculated concentration Quantitative result is within the linear range of the assay.
Yes	Yes	$> 2 \times 10^7$	Valid result: HBV DNA detected, $> 2 \times 10^7$ IU/ml Quantitation not possible since the quantitative result is above the linear range of the assay.*
No	Yes	–	Valid result: No HBV DNA is detectable.†
No	No	–	Invalid result: No result can be concluded.‡

* If quantitation is desired, dilute the sample with HBV-free plasma 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 HBV 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 (HBV RG/TM QS 1–5) in the *artus* HBV 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 5 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).

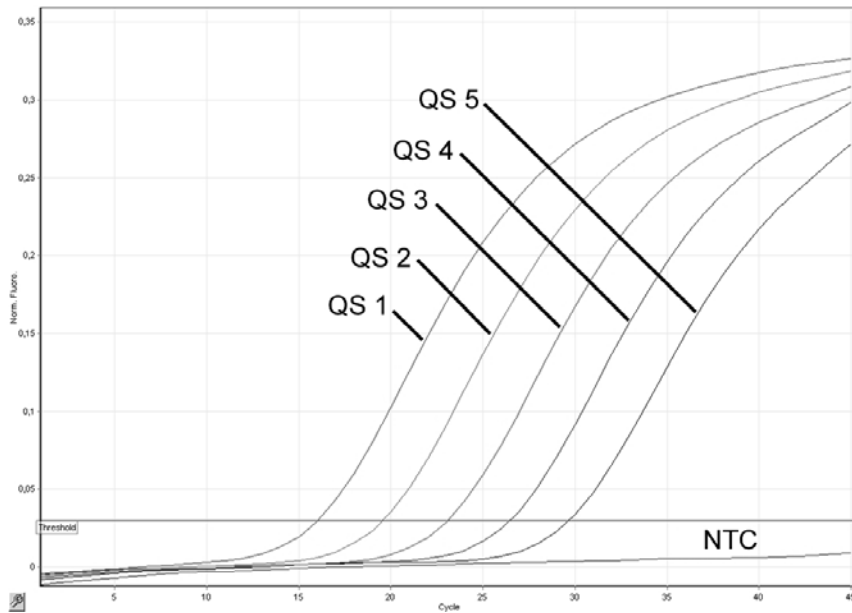
Conversion factor

1 IU/ml corresponds to 8.21 copies/ml for detection of HBV DNA on the Rotor-Gene Q. The conversion factor is an approximation based on an average factor across the assay's dynamic range.

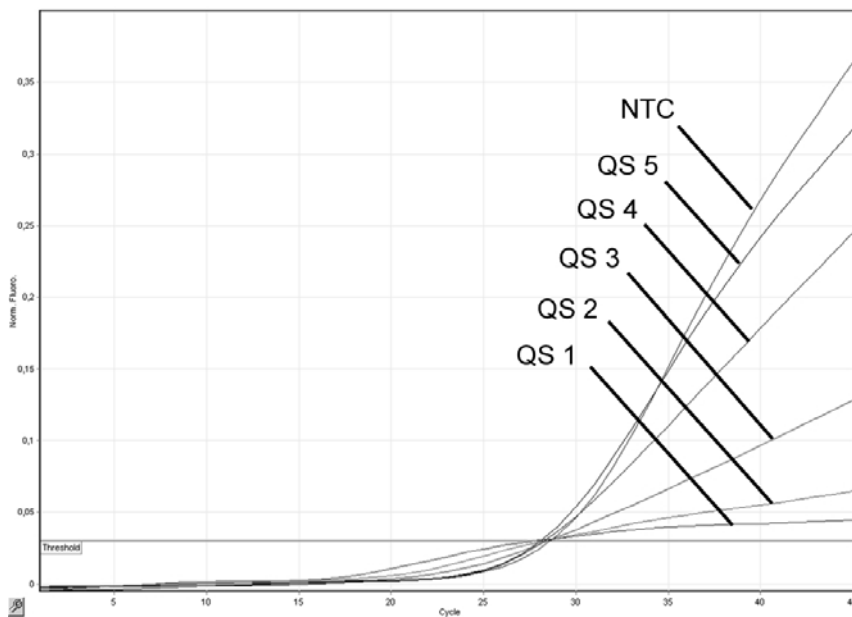
* The standard has been calibrated using the International HBV standard (WHO).

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

Examples of positive and negative PCR reactions



Detection of the quantitation standards (HBV RG/TM QS 1–5) in fluorescence channel Cycling Green. NTC: No template control (negative control).



Detection of the internal control (IC) in fluorescence channel Cycling Yellow with simultaneous amplification of the quantitation standards (HBV RG/TM QS 1–5). NTC: No template control (negative control).

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