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artus® HBV RG PCR Kit Handbook

Σ 24 (catalog no. 4506263)

Σ 96 (catalog no. 4506265)

Version 1

IVD

Quantitative in vitro diagnostics

For use with Rotor-Gene® Q Instruments

CE 0197

REF 4506263, 4506265

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Contents

Kit Contents

***artus* HBV RG PCR Kit**			**(24)**	**(96)**
Catalog no.			**4506263**	**4506265**
Number of reactions			**24**	**96**
Blue	HBV RG/TM Master		2 x 12 reactions	8 x 12 reactions
Red	HBV RG/TM QS 1* (1 x 10^5 IU/µl)	QS	200 µl	200 µl
Red	HBV RG/TM QS 2* (1 x 10^4 IU/µl)	QS	200 µl	200 µl
Red	HBV RG/TM QS 3* (1 x 10^3 IU/µl)	QS	200 µl	200 µl
Red	HBV RG/TM QS 4* (1 x 10^2 IU/µl)	QS	200 µl	200 µl
Red	HBV RG/TM QS 5* (1 x 10^1 IU/µl)	QS	200 µl	200 µl
Green	HBV RG/TM IC†	IC	1000 µl	2 x 1000 µl
White	Water (PCR grade)		1000 µl	1000 µl
	Handbook	HB	1	1

* Quantitation standard.

† Internal control.

Symbols

<N>

Contains reagents sufficient for <N> tests

Use by

IVD

In vitro diagnostic medical device

REF

Catalog number

LOT

Lot number

MAT

Material number

COMP	Components
CONT	Contains
NUM	Number
GTIN	Global Trade Item Number
	Temperature limitation
	Manufacturer
	Consult instructions for use
	Important note

Storage

The components of the *artus* HBV RG PCR Kit should be stored at –15°C to –30°C and are stable until the expiration date stated on the label. Repeated thawing and freezing (>2 x) should be avoided, as this may reduce assay sensitivity. If the reagents are to be used only intermittently, they should be frozen in aliquots. Storage at 2–8°C should not exceed a period of 5 hours.

Intended Use

The *artus* HBV RG PCR Kit is an in vitro nucleic acid amplification test for the quantitation of hepatitis B virus (HBV) DNA in human plasma. This diagnostic test kit utilizes the polymerase chain reaction (PCR) and is configured for use with Rotor-Gene Q Instruments.

Product Use Limitations

All reagents may exclusively be used in in vitro diagnostics.

The product is to be used by personnel specially instructed and trained in the in vitro diagnostics procedures only.

Strict compliance with the user manual is required for optimal PCR results.

Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.

Although rare, mutations within the highly conserved regions of the viral genome covered by the kit's primers and/or probe may result in underquantitation or failure to detect the presence of the virus in these cases. Validity and performance of the assay design are revised at regular intervals.

Warnings and Precautions

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN® kit and kit component.

Discard sample and assay waste according to your local safety regulations.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of *artus* HBV RG PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The *artus* HBV RG PCR Kit constitutes a ready-to-use system for the detection of HBV DNA using polymerase chain reaction (PCR) on Rotor-Gene Q Instruments. The HBV RG/TM Master contain reagents and enzymes for the specific amplification of a 134 bp region of the HBV genome, and for the direct detection of the specific amplicon in fluorescence channel Cycling Green of the Rotor-Gene Q or Rotor-Gene 6000, or Cycling A.FAM™ of the Rotor-Gene 3000.

In addition, the *artus* HBV RG PCR Kit contains a second heterologous amplification system to identify possible PCR inhibition. This is detected as an internal control (IC) in fluorescence channel Cycling Yellow of the Rotor-Gene Q or Rotor-Gene 6000, or A.JOE™ of the Rotor-Gene 3000. The detection limit of the analytical HBV PCR (see "Analytical sensitivity", page 8) is not reduced. External positive controls (HBV RG/TM QS 1–5) are supplied, which allow the determination of the amount of viral DNA. For further information, see "Quantitation", page 20.

Principle

Pathogen detection by the polymerase chain reaction (PCR) is based on the amplification of specific regions of the pathogen genome. In real-time PCR the amplified product is detected via fluorescent dyes. These are usually linked to oligonucleotide probes that bind specifically to the amplified product. Monitoring the fluorescence intensities during the PCR run (i.e., in real time) allows the detection and quantitation of the accumulating product without having to re-open the reaction tubes after the PCR run.*

Pathogen information

Hepatitis B virus (HBV) is mainly transmitted via blood or blood products. However, sexual, oral, and perinatal infections are also possible. Following a general malaise, including appetite loss, vomiting, and abdominal problems, about 10–20% of patients develop fever, exanthema (skin rash), as well as rheumatoid joint and muscle problems. 2–14 days later jaundice develops, which may be accompanied by itching. Fulminant hepatitis occurs in about 1% of all infected patients and is frequently fatal. 5–10% of hepatitis B patients develop chronic liver inflammation, which can progress to cirrhosis of the liver or primary liver cell carcinoma.

* Mackay, I.M. (2004) Real-time PCR in the microbiology laboratory. Clin. Microbiol. Infect. **10**, 190.

Performance characteristics

Analytical sensitivity

The analytical detection limit as well as the analytical detection limit in consideration of the purification (sensitivity limits) were assessed for the *artus* HBV RG PCR Kit. The analytical detection limit in consideration of the purification is determined using HBV-positive clinical specimens in combination with a particular extraction method. In contrast, the analytical detection limit is determined without clinical specimens and independent from the selected extraction method, using a standard of known concentration.

To determine the analytical sensitivity of the *artus* HBV RG PCR Kit, a dilution series was set up from 10 to nominal 0.0003 HBV IU/µl and analyzed with the *artus* HBV RG PCR Kit on Rotor-Gene Instruments. Testing was carried out on 3 different days on 8 replicates. The results were determined by a probit analysis. The analytical detection limit of the *artus* HBV RG PCR Kit in combination with Rotor-Gene 3000 is 0.02 IU/µl ($p = 0.05$). This means that there is a 95% probability that 0.02 IU/µl will be detected.

Equivalence between the Rotor-Gene 3000 and the Rotor-Gene Q/6000 was shown on the basis of technical specifications confirmed by analytical performance comparison. Probit analyses were performed on both systems in parallel. The analytical detection limit on the Rotor-Gene Q/6000 lies within the confidence interval of the Rotor-Gene 3000. Therefore, the *artus* HBV RG PCR Kit can be used for detection of HBV DNA on the Rotor-Gene Q/6000 with similar sensitivity.

The analytical sensitivity in consideration of the purification (QIAamp® DSP Virus Kit) of the *artus* HBV RG PCR Kit was determined using a dilution series of the 1st International HBV standard (WHO) from 158 to nominal 0.4 HBV IU/ml spiked in clinical plasma specimens. These were subjected to DNA extraction using the QIAamp DSP Virus Kit (extraction volume: 0.5 ml, elution volume: 26 µl). Each of the 7 dilutions was analyzed with the *artus* HBV RG PCR Kit on 3 different days on 8 replicates. The results were determined by a probit analysis. A graphical illustration of the probit analysis is shown in Figure 1. The analytical detection limit in consideration of the purification of the *artus* HBV RG PCR Kit in combination with the Rotor-Gene 3000 is 3.8 IU/ml ($p = 0.05$). This means that there is a 95% probability that 3.8 IU/ml will be detected.

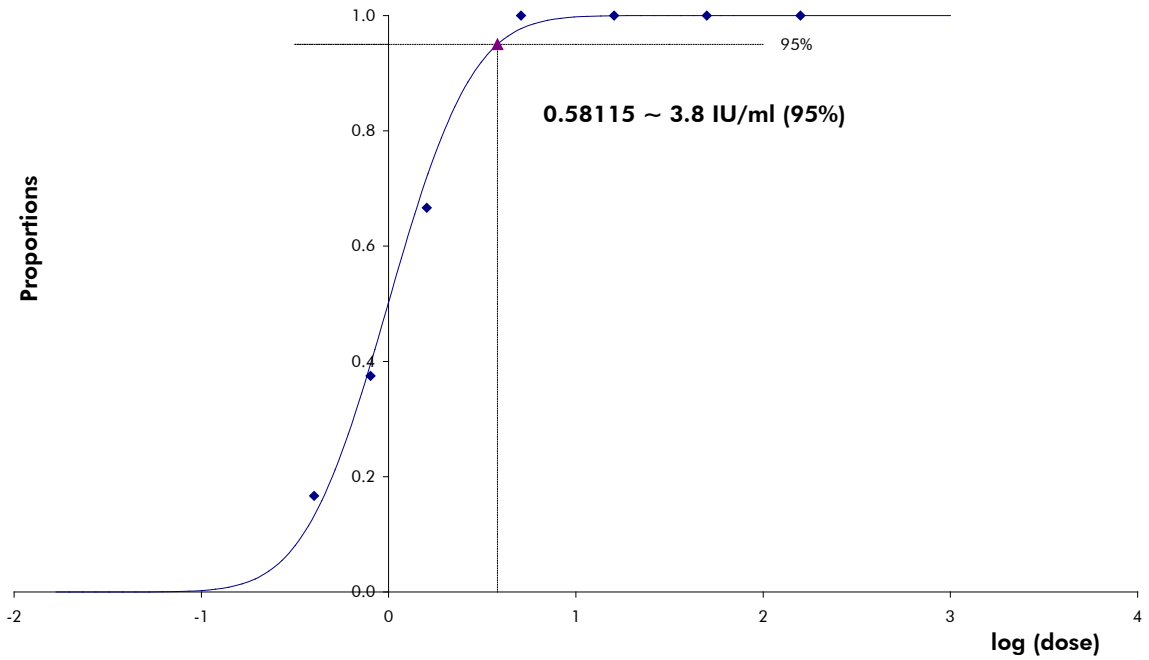

Figure 1. Probit analysis: HBV (Rotor-Gene 3000). Analytical sensitivity in consideration of the purification (QIAamp DSP Virus Kit, QIAGEN) of the *artus* HBV RG PCR Kit on the Rotor-Gene 3000.

Specificity

The specificity of the *artus* HBV RG PCR Kit is first and foremost ensured by the selection of the primers and probes, as well as the selection of stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences published in gene banks by sequence comparison analysis. The detectability of all relevant genotypes has thus been ensured by a database alignment and by a PCR run on Rotor-Gene Instruments with the following genotypes (see Table 1).

Table 1. Testing of the specificity of relevant genotypes

Virus	Genotype	Source	HBV (Cycling Green or A.FAM)	Internal control (Cycling Yellow or A. JOE)
HBV	A (USA)	Teragenix*	+	+
HBV	B (Indonesia)	Teragenix	+	+
HBV	C (Indonesia)	Teragenix	+	+
HBV	C (Venezuela)	Teragenix	+	+
HBV	D (USA)	Teragenix	+	+
HBV	E (Cote D'Ivoire)	Teragenix	+	+
HBV	F (Venezuela)	Teragenix	+	+
HBV	G (USA)	Teragenix	+	+
HBV	H (Nicaragua)	Teragenix	+	+

* Teragenix Corporation, Florida, USA.

For further specificity testing, HBV strains with known sequence differences in the pre-core region of the HBV genome (HBV Pre-Core Mutant Panel, Teragenix, Florida, USA) were used. All 9 pre-core mutant strains of this panel could be detected using the *artus* HBV RG PCR Kit.

Moreover, the specificity was validated with 100 different HBV negative plasma samples. These did not generate any signals with the HBV specific primers and probes, which are included in the HBV RG/TM Master.

A potential cross-reactivity of the *artus* HBV RG PCR Kit was tested using the control group listed in Table 2 (page 11). None of the tested pathogens has been reactive. No cross-reactivities appeared with mixed infections.

Linear range

The linear range (analytical measurement) of the *artus* HBV RG PCR Kit was determined by analyzing a dilution series of a HBV quantitation standard ranging from 1 x 10^8 IU/µl to 1 x 10^{-2} IU/µl. The dilution series was calibrated against the WHO 1st International HBV DNA Standard.

Each dilution was tested in replicates (n = 8 for concentrations ≥1 x 10^0 IU/µl; n = 16 for concentrations <1 x 10^0 IU/µl) using the *artus* HBV RG PCR Kit on Rotor-Gene Instruments.

Table 2. Testing the specificity of the kit with potentially cross-reactive pathogens

Control group	HBV (Cycling Green or Cycling A.FAM)	Internal control (Cycling Yellow or Cycling A.JOE)
Human herpesvirus 1 (Herpes simplex virus 1)	–	+
Human herpesvirus 2 (Herpes simplex virus 2)	–	+
Human herpesvirus 3 (Varicella-zoster virus)	–	+
Human herpesvirus 4 (Epstein-Barr virus)	–	+
Human herpesvirus 5 (Cytomegalovirus)	–	+
Human herpesvirus 6	–	+
Human immunodeficiency virus 1	–	+
Hepatitis A virus	–	+
Hepatitis C virus	–	+
Parvovirus B19	–	+
Yellow fever virus	–	+
Human T cell leukemia virus type 1 and type 2	–	+
Coxsackie virus B3	–	+
Dengue virus 1–4	–	+
Escherichia coli	–	+

The linear range of the *artus* HBV RG PCR Kit was determined to cover concentrations from 0.02 IU/µl to at least 1 x 10^8 IU/µl (Figure 2).

Under the assumption that the QIAamp DSP Virus Kit is used for DNA extraction, the *artus* HBV RG PCR Kit covers a linear range from 1.1 IU/ml to at least 4 x 10^9 IU/ml.

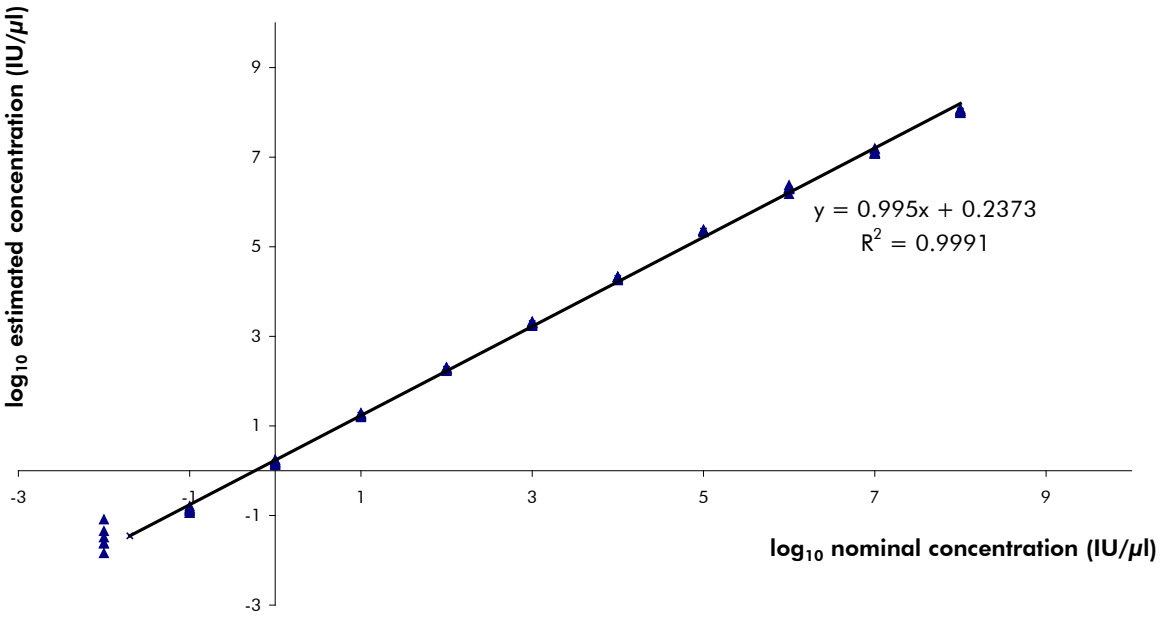

Figure 2. Linear Range of the *artus* HBV RG PCR Kit. Calculation of the linear range. The straight line was determined by a linear regression of the $\log_{10}$ calculated concentrations with the $\log_{10}$ nominal concentrations. The equation of the regression line is included in the figure.

Precision

The precision data of the *artus* HBV RG PCR Kit allow determination of the total variance of the assay. The total variance consists of the intra-assay variability (variability of multiple results of samples of the same concentration within one experiment), the inter-assay variability (variability of multiple results of the assay generated on different instruments of the same type by different operators within one laboratory) and the inter-batch variability (variability of multiple results of the assay using various batches). The data obtained were used to determine the standard deviation, the variance and the coefficient of variation for the pathogen specific and the internal control PCR.

Precision data of the *artus* HBV RG PCR Kit were collected using the Quantitation Standard of the lowest concentration (QS 5; 10 IU/µl). Testing was performed with 8 replicates. The precision data were calculated on basis of the C_T values of the amplification curves (C_T: threshold cycle, see Table 3, page 13). In addition, precision data for quantitative results in IU/µl were determined using the corresponding C_T values (Table 4). Based on these results, the overall statistical spread of any given sample with the mentioned concentration is 1.29% (C_T) or 8.99% (concentration), and 1.87% (C_T) for the detection of the internal control. These values are based on the totality of all single values of the determined variabilities.

Table 3. Precision data on basis of the C_T values

	Standard deviation	Variance	Coefficient of variation (%)
Intra-assay variability: HBV RG/TM QS 5	0.09	0.01	0.32
Intra-assay variability: Internal control	0.10	0.01	1.06
Inter-assay variability: HBV RG/TM QS 5	0.14	0.02	0.49
Inter-assay variability: Internal control	0.29	0.08	1.00
Inter-batch variability: HBV RG/TM QS 5	0.38	0.15	1.39
Inter-batch variability: Internal control	0.62	0.39	2.23
Total variance: HBV RG/TM QS 5	0.36	0.13	1.29
Total variance: Internal control	0.52	0.27	1.87

Table 4. Precision data on basis of the quantitative results (in IU/µl)

	Standard deviation	Variance	Coefficient of variation (%)
Intra-assay variability: HBV RG/TM QS 5	0.93	0.87	9.28
Inter-assay variability: HBV RG/TM QS 5	0.79	0.63	7.92
Inter-batch variability: HBV RG/TM QS 5	1.03	1.05	10.21
Total variance: HBV RG/TM QS 5	0.90	0.81	8.99

Robustness

The verification of the robustness allows the determination of the total failure rate of the *artus* HBV RG PCR Kit. To verify the robustness, 100 HBV negative samples of plasma were spiked with 0.05 IU/µl elution volume of HBV control DNA (approximately threefold concentration of the analytical sensitivity limit). After extraction using the QIAamp DSP Virus Kit (see "DNA isolation", page 18), these samples were analyzed with the *artus* HBV RG PCR Kit. For all HBV samples the failure rate was 0%. In addition, the robustness of the internal control was assessed by purification and analysis of 100 HBV negative plasma samples. The total failure rate was 0%. Inhibitions were not observed. Thus, the robustness of the *artus* HBV RG PCR Kit is ≥99%.

Reproducibility

Reproducibility data permit a regular performance assessment of the *artus* HBV RG PCR Kit as well as an efficiency comparison with other products. These data are obtained by the participation in established proficiency programs.

Diagnostic evaluation

In a study in 2 independent laboratories, the *artus* HBV RG PCR Kit was compared to the COBAS® TaqMan® HBV Assay. For this purpose, 287 retrospective and prospective plasma specimens were tested.

HBV DNA for testing the *artus* HBV RG PCR Kit was isolated using the QIAamp DSP Virus Kit, and analysis was carried out on the Rotor-Gene 3000 Instrument. For comparative testing with the COBAS TaqMan HBV Assay, HBV DNA was isolated according to the instructions of the manufacturer provided in the package insert. The results obtained by using the *artus* HBV RG PCR Kit were compared to those of the COBAS TaqMan HBV Assay.

In comparison to the results collected with the COBAS TaqMan HBV Assay as a reference assay, a diagnostic sensitivity of the *artus* HBV RG PCR Kit of 100% and a diagnostic specificity of 97% was determined for the totality of all plasma samples. These results are represented in Table 5.

Table 5. Results of the comparative validation study

		COBAS TaqMan HBV Assay		
		+	–	**Total**
***artus* HBV RG PCR Kit**	+	186	3	189
	–	0	98	98

Further testing of the 3 discordant samples confirmed the results of the *artus* HBV RG PCR Kit. Therefore it can be assumed that the discrepancy is based on a higher sensitivity of the *artus* HBV RG PCR Kit.

The correlation of the quantitative results of both test systems was analyzed by linear regression. The results of both kits are shown in comparison in Figure 3.

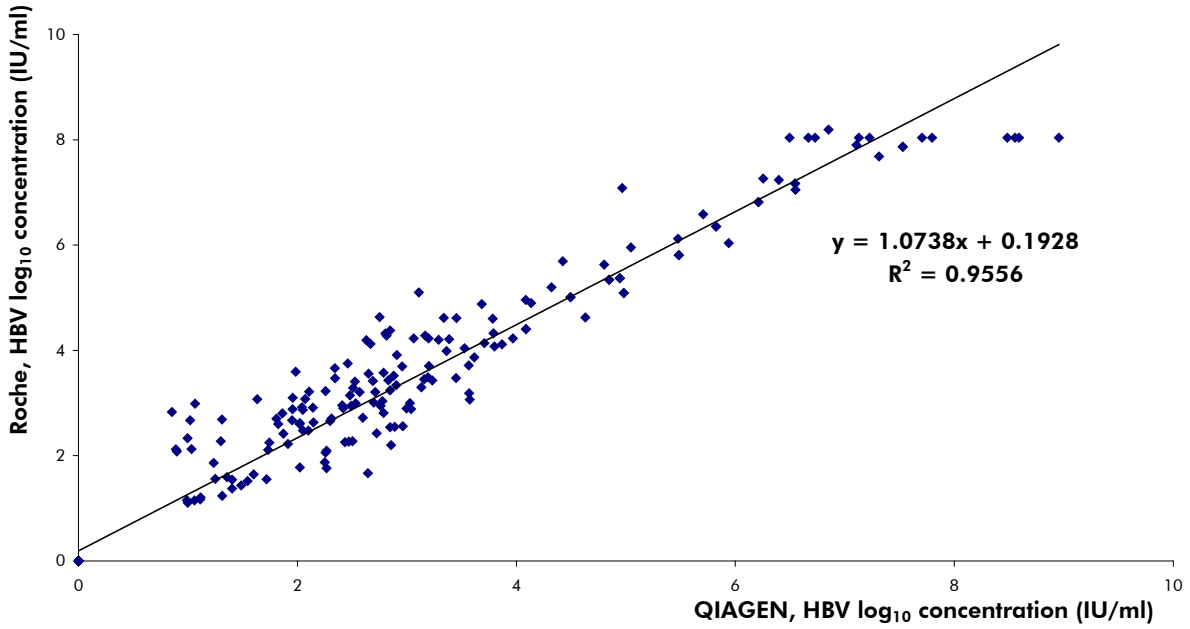

Figure 3. Comparison of the COBAS TaqMan HBV Assay (Roche, HBV; with sample purification using the High Pure system) with the *artus* HBV RG PCR Kit (QIAGEN, HBV; with sample purification using the QIAamp DSP Virus Kit). Correlation of quantitative results from both test systems (Table 5) was analyzed by linear regression. The results from both kits are shown in an XY (scatter) plot with log–log scale.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- DNA isolation kit (see "DNA isolation", page 18)
- Pipets (adjustable)*
- Sterile pipet tips with filters
- Vortex mixer*
- Benchtop centrifuge* with rotor for 2 ml reaction tubes
- Rotor-Gene Q or Rotor-Gene Instrument* with fluorescence channels for Cycling Green and Cycling Yellow or with fluorescence channels for Cycling A.FAM and Cycling A.JOE
- Rotor-Gene Q software version 1.7.94 (Rotor-Gene 6000 software version 1.7.65, 1.7.87, 1.7.94; Rotor-Gene 3000 software version 6.0.23) or higher
- Strip Tubes and Caps, 0.1 ml, for use with 72-well rotor (cat. no. 981103 or 981106)
- Alternatively: PCR Tubes, 0.2 ml, for use with 36-well rotor (cat. no. 981005 or 981008)
- Cooling block (Loading Block 72 x 0.1 ml Tubes, cat. no. 9018901, or Loading Block 96 x 0.2 ml Tubes, cat. no. 9018905)

* Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

Important Notes

General precautions

The user should always pay attention to the following:

- Use sterile pipet tips with filters.
- Store and extract positive materials (specimens, positive controls, and amplicons) separately from all other reagents, and add them to the reaction mix in a spatially separated facility.
- Thaw all components thoroughly at room temperature (15–25°C) before starting an assay.
- When thawed, mix the components (by pipetting repeatedly up and down or by pulse vortexing) and centrifuge briefly.
- Work quickly and keep components on ice or in the cooling block (72/96-well loading block).

Specimen collection, storage, and transport

ⓘ All samples must be treated as potentially infectious material.

ⓘ Current studies refer to EDTA or citrate plasma as the most suitable sample materials for HBV detection. Therefore, we recommend the use of these materials with the *artus* HBV RG PCR Kit.

The internal validation of the *artus* HBV RG PCR Kit was performed using human EDTA plasma samples. Other sample materials are not validated. Please use only the recommended nucleic acid isolation kit (see "DNA isolation", page 18) for sample preparation.

Using certain sample materials, particular instructions regarding collection, transport, and storage have to be strictly observed.

Specimen collection

Each blood withdrawal causes an injury of blood vessels (arteries, veins, capillaries). Only innocuous and sterile material should be used. For blood withdrawal appropriate disposables are available. For vein punctures, capillary needles that are too fine should not be used. Venous blood withdrawal should be carried out on the appropriate parts of the elbow bend, the forearm, or the back of the hand. Blood has to be withdrawn with standard specimen collection tubes (red cap, Sarstedt or equivalent tube of another manufacturer). A volume of 5–10 ml EDTA blood should be withdrawn. Tubes should be mixed over-head directly after sample collection (8 x, do not agitate).

ⓘ Samples from heparinized humans must not be used (see “Interfering substances”, unterhalb).

Sample storage

Whole blood should be separated into plasma and cellular components by centrifugation for 20 minutes at 800–1600 x *g* within 6 hours. The isolated plasma must be transferred into sterile polypropylene tubes. The sensitivity of the assay can be reduced if you freeze the samples as a matter of routine or store them for a longer period of time. Virus encapsulated DNA is stable for days if stored at 4°C, for weeks if stored at –20°C, and even for months and years when stored at –70°C.*

Sample transport

Sample material should be transported in a shatterproof transport container as a matter of principle. Thus, a potential danger of infection due to a leakage of sample can be avoided. The samples should be transported following the local and national instructions for the transport of pathogen material.†

The samples should be shipped within 6 hours. We do not recommend to store the samples where they have been collected. It is possible to ship the samples by mail, following the legal instructions for the transport of pathogen material. We recommend sample transport with a courier. The blood samples should be shipped cooled (2–8°C) and the separated plasma deep frozen (–15 to –30°C).

Interfering substances

Elevated levels of bilirubin (≤15 mg/dl) and lipids (≤800 mg/dl) and hemolytic samples do not influence the system. Heparin (≥10 IU/ml) affects the PCR. Samples that have been collected in tubes containing heparin as an anticoagulant should not to be used. Also, samples of heparinized patients must not be used.

DNA isolation

The QIAamp DSP Virus Kit (QIAGEN, cat. no. 60704) is validated for viral DNA purification from human plasma for use with the *artus* HBV RG PCR Kit. Carry out the viral DNA purification according to the instructions in the *QIAamp DSP Virus Kit Handbook*.

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

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

ⓘ The use of carrier RNA is critical for the extraction efficiency and, consequently, for DNA/RNA yield. To increase the stability of the carrier RNA provided with the QIAamp DSP Virus Kit, we recommend to proceed according to the information about the reconstitution and storage of the carrier RNA given in the instruction manual ("Preparing reagents and buffers").

ⓘ The internal control of the *artus* HBV RG PCR Kit can be used directly in the isolation procedure (see "Internal control", below). Make sure to co-process a negative plasma sample in the purification. Its corresponding internal control signal serves as a basis for assessment of the purification.

Internal control

An internal control (HBV RG/TM IC) is supplied. This allows the user both to control the DNA isolation procedure and to check for possible PCR inhibition. For this application, add the internal control to the isolation at a ratio of 0.1 µl per 1 µl elution volume. For example, using the QIAamp DSP Virus Kit, the DNA is eluted in 60 µl Elution Buffer (AVE). Hence, 6 µl of the internal control should be added initially. The quantity of internal control used depends only on the elution volume.

ⓘ The internal control and carrier RNA (see "DNA isolation", page 18) should be added only to the mixture of lysis buffer and sample material or directly to the lysis buffer.

The internal control must not be added to the sample material directly. If added to the lysis buffer please note that the mixture of internal control and lysis buffer–carrier RNA has to be prepared freshly and used immediately (storage of the mixture at room temperature or in the fridge for only a few hours may lead to internal control failure and a reduced extraction efficiency).

ⓘ Do not add the internal control and the carrier RNA to the sample material directly.

To consider a purification successful, the C_T value of the internal control of a negative plasma sample that has been processed during purification (QIAamp DSP Virus Kit) has to reach $C_T = 29 \pm 3$ (threshold: 0.03) using Rotor-Gene Q Instruments. The stated spreading is based on the variance of the instrument and the purification. A higher deviation points to a purification problem. In this case the purification has to be checked and, if necessary, validated a second time. If you have any further questions or if you encounter problems, please contact QIAGEN Technical Services.

The internal control can optionally be used exclusively to check for possible PCR inhibition. For this application, add the internal control directly to the HBV RG/TM Master, as described in step 2b of the protocol (page 22).

Setting the threshold for the PCR analysis

The optimal threshold settings for a given combination of Rotor-Gene Q instrument and *artus* RG PCR Kit should be set empirically by testing each individual combination since it is a relative value depending on the overall diagnostic workflow. As a starting point, 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 enclosed quantitation standards (HBV RG/TM QS 1–5) 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 as standards with the specified concentrations (see the instrument user manual).

ⓘ 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:

$$\text{Result (IU/ml)} = \frac{\text{Result (IU/µl)} \times \text{Elution Volume (µl)}}{\text{Sample Volume (ml)}}$$

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 standard has been calibrated using the 1st International HBV standard (WHO).

Protocol: PCR and Data Analysis

Important points before starting

- Before beginning the procedure, read "Important Notes", pages 17–20.
- Take time to familiarize yourself with the Rotor-Gene Q before starting the protocol. See the instrument user manual.
- Make sure that at least one quantitation standard as well as one negative control (Water, PCR grade) are included per PCR run. To generate a standard curve, use all 5 quantitation standards supplied (HBV RG/TM QS 1–5) for each PCR run.

Things to do before starting

- Make sure that the cooling block (accessory of the Rotor-Gene Q Instrument) is precooled to 2–8°C.
- Before each use, all reagents need to be thawed completely, mixed (by repeated up and down pipetting or by quick vortexing), and centrifuged briefly.

Procedure

1. Place the desired number of PCR tubes into the adapters of the cooling block.

2. If you are using the internal control to monitor the DNA isolation procedure and to check for possible PCR inhibition, follow step 2a. If you are using the internal control exclusively to check for PCR inhibition, follow step 2b.

2a. The internal control has already been added to the isolation (see "Internal control", page 19). In this case, prepare a master mix according to Table 6.

Table 6. Preparation of the master mix (internal control used to monitor DNA isolation and check for PCR inhibition)

Number of samples	1	12
HBV RG/TM Master	30 µl	360 µl
HBV RG/TM IC	0 µl	0 µl each
Total volume	**30 µl**	**360 µl each**

2b. The internal control must be added directly to the HBV RG/TM Master. In this case, prepare a master mix according to Table 7.

The reaction mix typically contains all of the components needed for PCR except the sample.

Table 7. Preparation of master mix (internal control used exclusively to check for PCR inhibition)

Number of samples	1	12
HBV RG/TM Master	30 µl	360 µl
HBV RG/TM IC	2 µl	24 µl
Total volume	**32 µl***	**384 µl***

* The volume increase caused by adding the internal control is neglected when preparing the PCR assay. The sensitivity of the detection system is not impaired.

3. Pipet 30 µl of the master mix into each PCR tube. Then add 20 µl of the eluted sample DNA (see Table 8). Correspondingly, 20 µl of at least one of the quantitation standards (HBV RG/TM QS 1–5) must be used as a positive control and 20 µl of water (Water, PCR grade) as a negative control.

Table 8. Preparation of PCR assay

Number of samples	1	12
Master mix	30 µl	30 µl each
Sample	20 µl	20 µl each
Total volume	**50 µl**	**50 µl each**

4. Close the PCR tubes. Make sure that the locking ring (accessory of the Rotor-Gene Instrument) is placed on top of the rotor to prevent accidental opening of the tubes during the run.

5. **For the detection of HBV DNA, create a temperature profile according to the following steps.**

Setting the general assay parameters	**Figures 4, 5, 6**
Initial activation of the hot-start enzyme	**Figure 7**
Amplification of the DNA	**Figure 8**
Adjusting the fluorescence channel sensitivity	**Figure 9**
Starting the run	**Figure 10**

All specifications refer to the Rotor-Gene Q software version 1.7.94, Rotor-Gene 6000 software versions 1.7.65, 1.7.87, 1.7.94, and Rotor-Gene 3000 software version 6.0.23. Please find further information on programming Rotor-Gene Instruments in the instrument user manual. In the illustrations these settings are framed in bold black. Illustrations are included for Rotor-Gene Q Instruments. Where different values are required for the Rotor-Gene 3000, these differences are described in the text.

6. **First, open the "New Run Wizard" dialog box (Figure 4). Check the "Locking Ring Attached" box and click "Next".**

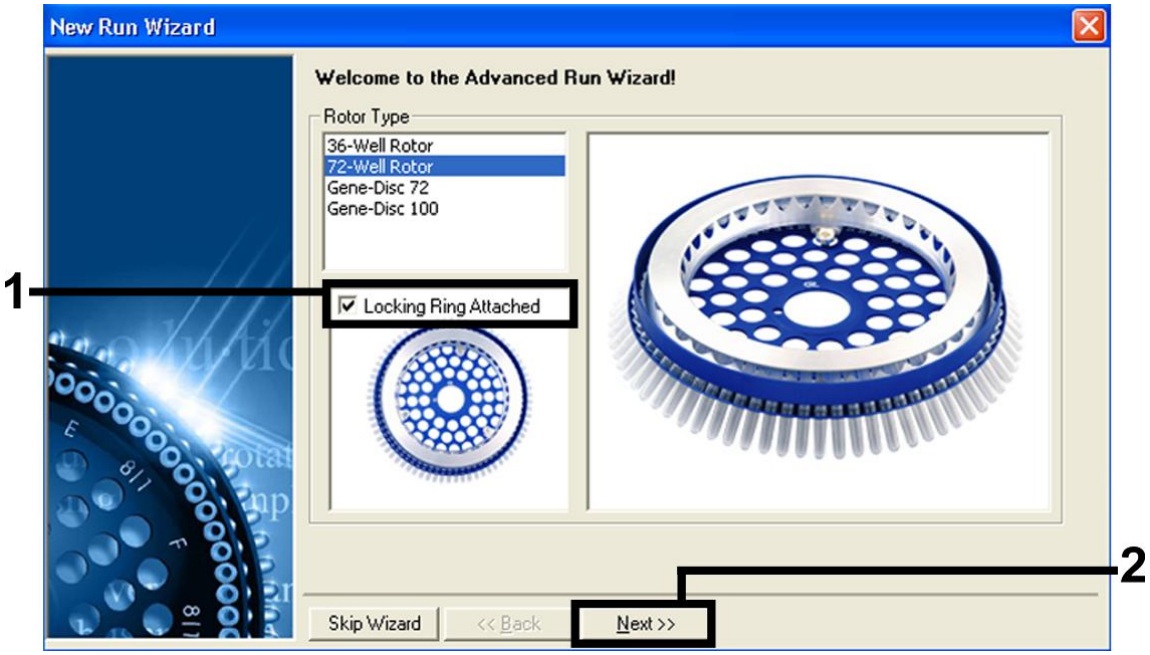

Figure 4. The "New Run Wizard" dialog box.

7. Select *50* for the PCR reaction volume and click "Next" (Figure 5).

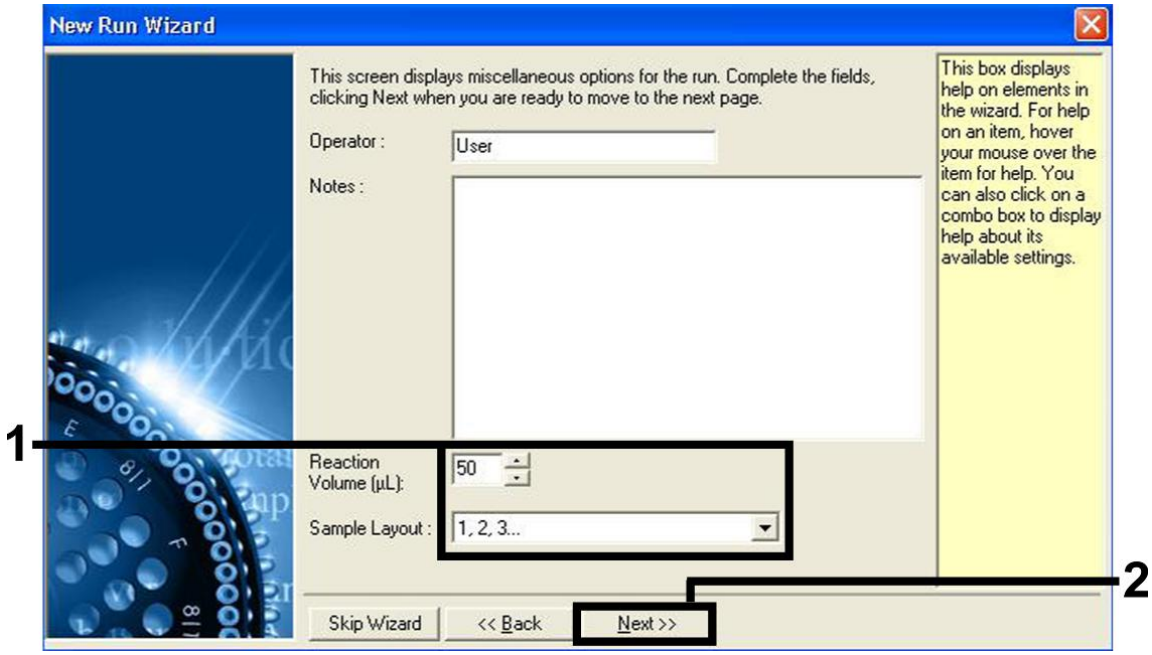

Figure 5. Setting the general assay parameters.

8. Click the "Edit Profile" button in the next "New Run Wizard" dialog box (Figure 6), and program the temperature profile as shown in Figures 6–8).

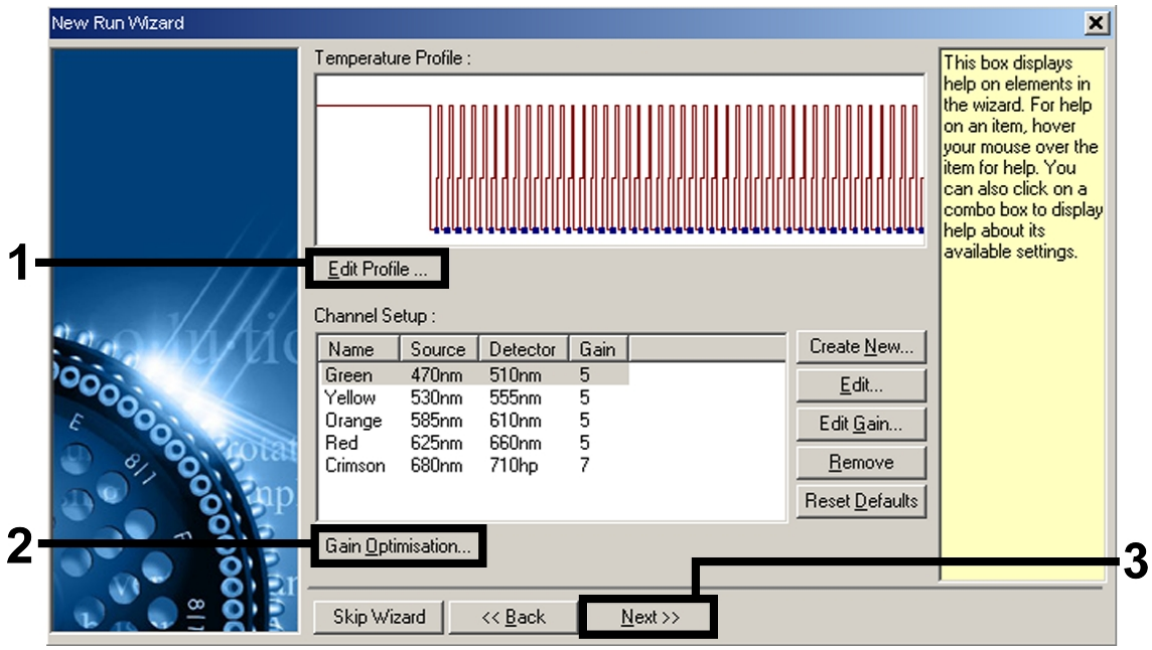

Figure 6. Editing the profile.

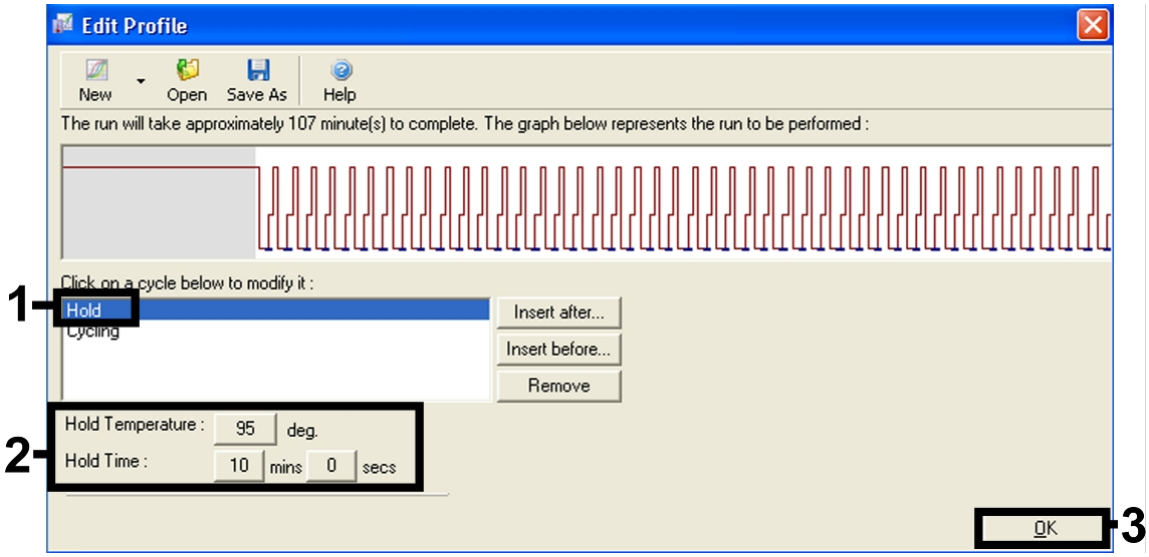

Figure 7. Initial activation of the hot-start enzyme.

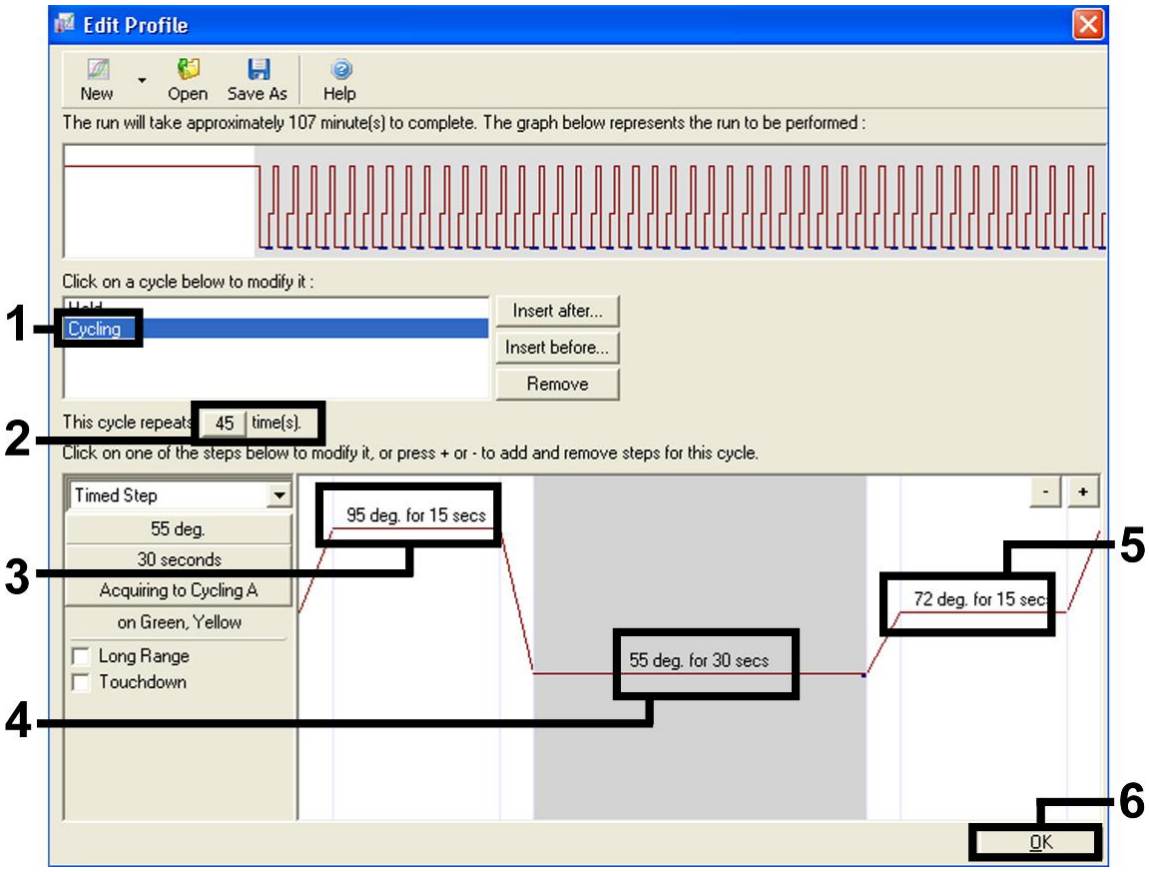

Figure 8. Amplification of the DNA. Note that, on the Rotor-Gene 3000, the software will define the fluorescence dyes as "FAM/Sybr, JOE".

9. **The detection range of the fluorescence channels has to be determined according to the fluorescence intensities in the PCR tubes. Click "Gain Optimisation" in the "New Run Wizard" dialog box (see Figure 6) to open the "Auto-Gain Optimisation Setup" dialog box. Set the calibration temperature to *55* to match the annealing temperature of the amplification program (Figure 9).**

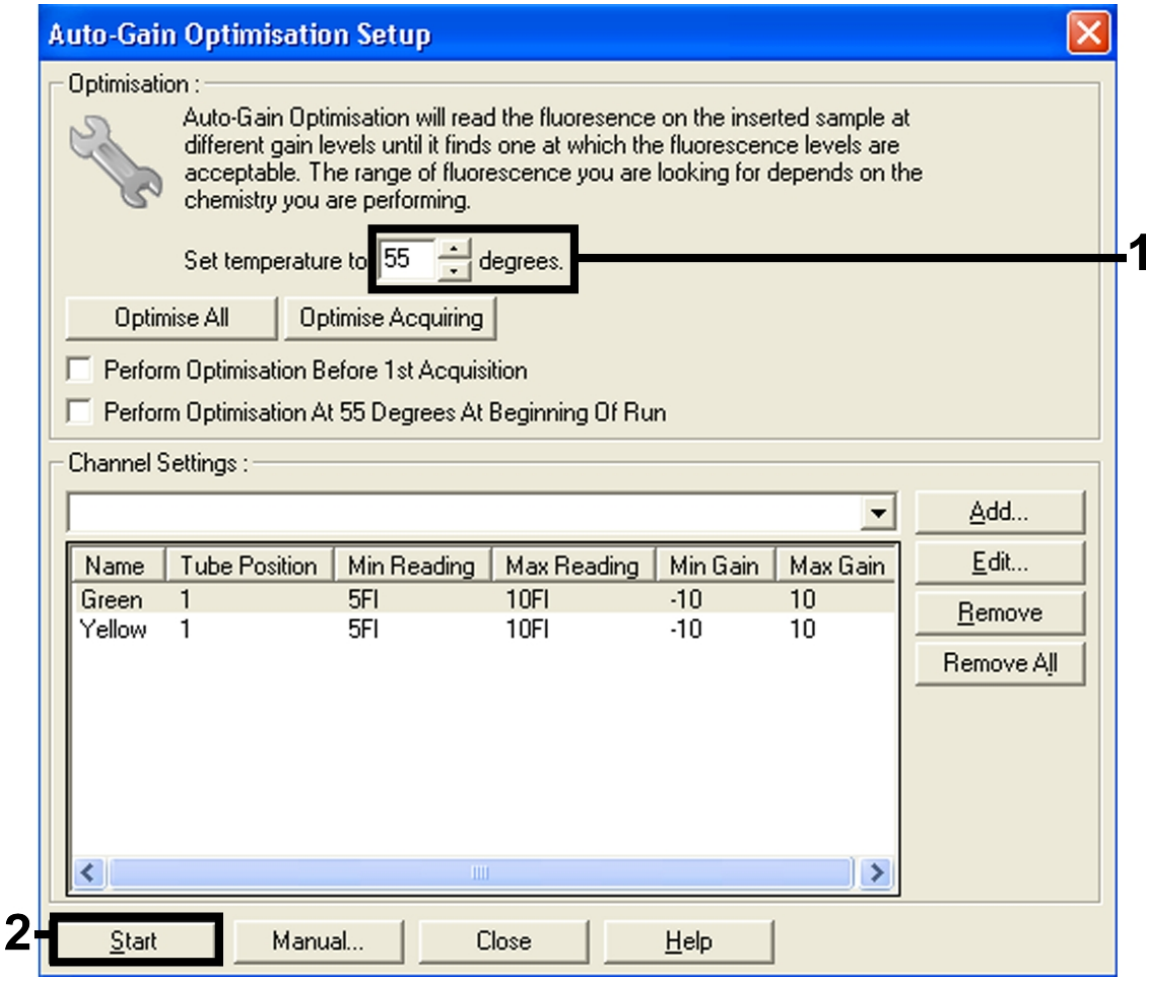

Figure 9. Adjusting the fluorescence channel sensitivity. Note that, on the Rotor-Gene 3000, the software will define the fluorescence dyes as "FAM/Sybr" and "JOE".

10. The gain values determined by the channel calibration are saved automatically and are listed in the last menu window of the programming procedure (Figure 10). Click "Start Run".

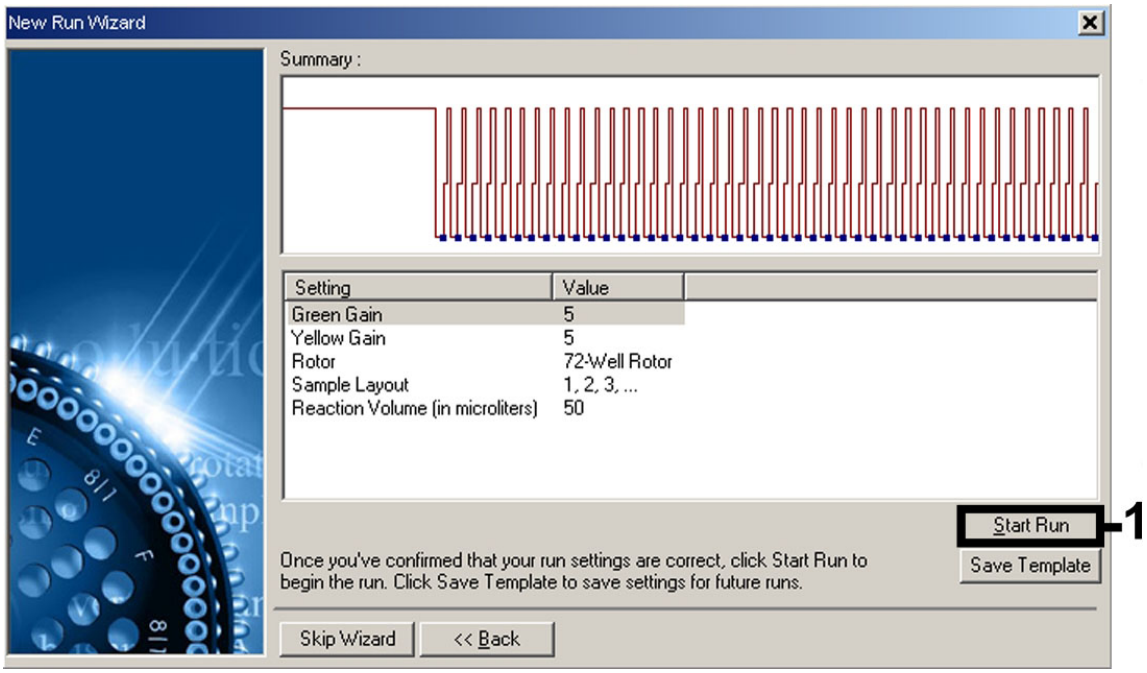

Figure 10. Starting the run. Note that, on the Rotor-Gene 3000, the software will define the fluorescence dyes as "FAM/Sybr" and "JOE".

11. After the run is finished, analyze the data. The following results (11a, 11b, and 11c) are possible.

Examples of positive and negative PCR reactions are given in Figure 11 and Figure 12.

11a. A signal is detected in fluorescence channel Cycling Green. The result of the analysis is positive: the sample contains HBV DNA.

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

ⓘ Note that, on the Rotor-Gene 3000, the relevant channels are Cycling A.FAM for the positive signal and Cycling A.JOE for the internal control.

11b. In fluorescence channel Cycling Green no signal is detected. At the same time, a signal from the internal control appears in the Cycling Yellow channel.
In the sample no HBV DNA is detectable. It can be considered negative.

In the case of a negative HBV PCR, the detected signal of the internal control rules out the possibility of PCR inhibition.

ⓘ Note that, on the Rotor-Gene 3000, the relevant channels are Cycling A.JOE for the internal control and lack of a signal for Cycling A.FAM.

11c. No signal is detected in the Cycling Green or in the Cycling Yellow channels.
No result can be concluded.

Information regarding error sources and their solution can be found in "Troubleshooting Guide", page 30.

ⓘ Note that, on the Rotor-Gene 3000, the relevant channels are Cycling A.FAM and Cycling A.JOE.

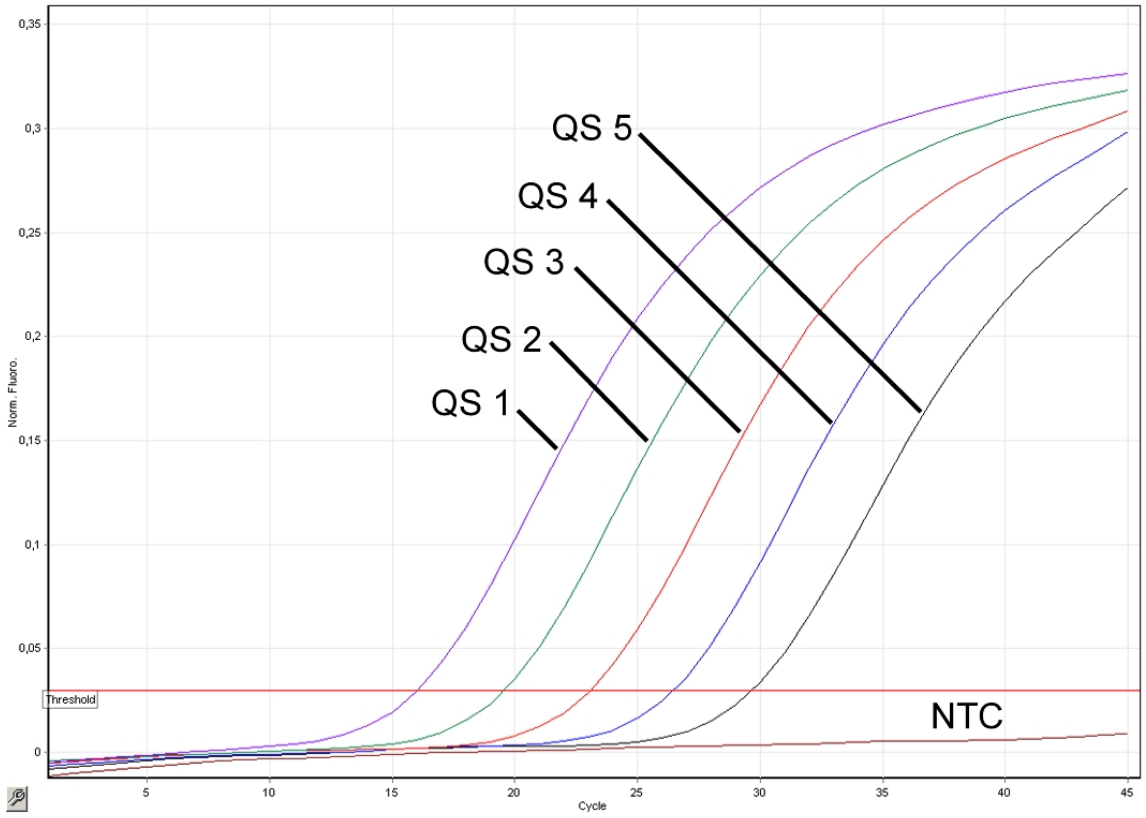

Figure 11. Detection of the quantitation standards (HBV RG/TM QS 1–5) in fluorescence channel Cycling Green. NTC: No template control (negative control).

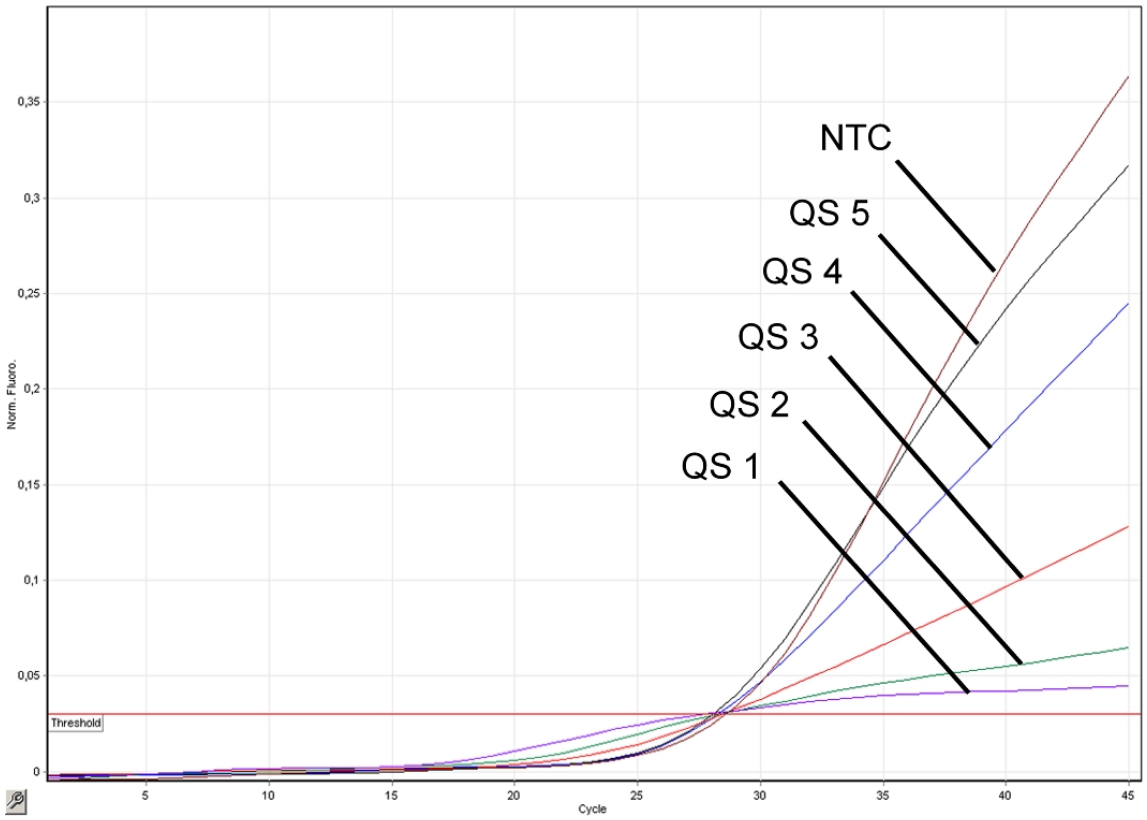

Figure 12. Detection of the internal control (IC) in fluorescence channel Cycling Yellow with simultaneous amplification of the quantitation standards (HBV RG/TM QS 1–5). NTC: No template control (negative control).

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

	Comments and suggestions
No signal with positive controls (HBV RG/TM QS 1–5) in fluorescence channel Cycling Green or Cycling A.FAM	
a) The selected fluorescence channel for PCR data analysis does not comply with the protocol	ⓘ For data analysis select the fluorescence channel Cycling Green or Cycling A.FAM for the analytical HBV PCR and the fluorescence channel Cycling Yellow or Cycling A.JOE for the internal control PCR.
b) Incorrect programming of the temperature profile of the Rotor-Gene Instrument	ⓘ Compare the temperature profile with the protocol. See "Protocol: PCR and Data Analysis", page 21.
c) Incorrect configuration of the PCR	ⓘ Check your work steps by means of the pipetting scheme, and repeat the PCR, if necessary. See "Protocol: PCR and Data Analysis", page 21.
d) The storage conditions for one or more kit components did not comply with the instructions given in "Storage" (page 5)	ⓘ Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
e) The *artus* HBV RG PCR Kit has expired	ⓘ Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.

	Comments and suggestions
Weak or no signal of the internal control of a negative plasma sample subjected to purification using the QIAamp DSP Virus Kit (C_T = 29 ± 3; threshold, 0.03) in fluorescence channel Cycling Yellow or Cycling A.JOE and simultaneous absence of a signal in channel Cycling Green or Cycling A.FAM	
a) The PCR conditions do not comply with the protocol	ⓘ Check the PCR conditions (see above) and repeat the PCR with corrected settings, if necessary.
b) The PCR was inhibited	ⓘ Make sure that you use the recommended isolation method and closely follow the manufacturer's instructions.
c) DNA was lost during extraction	ⓘ If the internal control was added to the extraction, an absent signal of the internal control can indicate the loss of DNA during the extraction. Make sure that you use the recommended isolation method (see "DNA isolation", page 18) and closely follow the manufacturer's instructions.
d) The storage conditions for one or more kit components did not comply with the instructions given in "Storage" (page 5)	ⓘ Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
e) The *artus* HBV RG PCR Kit has expired	ⓘ Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.

Comments and suggestions	
Signals with the negative controls in fluorescence channel Cycling Green or Cycling A.FAM of the analytical PCR	
a) Contamination occurred during preparation of the PCR	ⓘ Repeat the PCR with new reagents in replicates. ⓘ If possible, close the PCR tubes directly after addition of the sample to be tested. ⓘ Make sure to pipet the positive controls last. ⓘ Make sure that work space and instruments are decontaminated at regular intervals.
b) Contamination occurred during extraction	ⓘ Repeat the extraction and PCR of the sample to be tested using new reagents. ⓘ Make sure that work space and instruments are decontaminated at regular intervals.

References

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For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Ordering Information

Product	Contents	Cat. no.
artus HBV RG PCR Kit (24)	For 24 reactions: Master, 5 Quantitation Standards, Internal Control, Water (PCR grade)	4506263
artus HBV RG PCR Kit (96)	For 96 reactions: Master, 5 Quantitation Standards, Internal Control, Water (PCR grade)	4506265
QIAamp DSP Virus Kit — for purification of viral nucleic acids from human plasma for in vitro diagnostic purposes		
QIAamp DSP Virus Kit	For 50 preps: QIAamp MinElute® Spin Columns, Buffers, Reagents, Tubes, Column Extenders, and VacConnectors	60704
Rotor-Gene Q MDx — for IVD-validated real-time PCR analysis in clinical applications		
Rotor-Gene Q MDx 5plex System	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9002023
Rotor-Gene Q MDx 5plex Platform	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training not included	9002022
Rotor-Gene Q MDx 5plex HRM System	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9002033

Product	Contents	Cat. no.
Rotor-Gene Q MDx 5plex HRM Platform	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training not included	9002032
Rotor-Gene Q MDx 6plex System	Real-time PCR instrument with 6 channels (blue, green, yellow, orange, red, crimson), including laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9002043
Rotor-Gene Q MDx 6plex Platform	Real-time PCR instrument with 6 channels (blue, green, yellow, orange, red, crimson), including laptop comptuer, software, accessories, 1-year warranty on parts and labor, installation and training not included	9002042
Rotor-Gene Q MDx 2plex System	Real-time PCR cycler with 2 channels (green, yellow), laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9002003
Rotor-Gene Q MDx 2plex Platform	Real-time PCR cycler and High Resolution Melt analyzer with 2 channels (green, yellow) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training not included	9002002
Rotor-Gene Q MDx 2plex HRM System	Real-time PCR cycler and High Resolution Melt analyzer with 2 channels (green, yellow) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9002013

Product	Contents	Cat. no.
Rotor-Gene Q MDx 2plex HRM Platform	Real-time PCR cycler and High Resolution Melt analyzer with 2 channels (green, yellow) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training not included	9002012
Rotor-Gene Q — for outstanding performance in real-time PCR		
Rotor-Gene Q 5plex System	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9001640
Rotor-Gene Q 5plex Platform	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training not included	9001570
Rotor-Gene Q 5plex HRM System	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9001650
Rotor-Gene Q 5plex HRM Platform	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training not included	9001580
Rotor-Gene Q 6plex System	Real-time PCR instrument with 6 channels (blue, green, yellow, orange, red, crimson), including laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9001660

Product	Contents	Cat. no.
Rotor-Gene Q 6plex Platform	Real-time PCR instrument with 6 channels (blue, green, yellow, orange, red, crimson), including laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training not included	9001590
Rotor-Gene Q 2plex System	Real-time PCR cycler with 2 channels (green, yellow), laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9001620
Rotor-Gene Q 2plex Platform	Real-time PCR cycler and High Resolution Melt analyzer with 2 channels (green, yellow) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training not included	9001550
Rotor-Gene Q 2plex HRM System	Real-time PCR cycler and High Resolution Melt analyzer with 2 channels (green, yellow) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9001630
Rotor-Gene Q 2plex HRM Platform	Real-time PCR cycler and High Resolution Melt analyzer with 2 channels (green, yellow) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training not included	9001560
Rotor-Gene Q accessories		
Loading Block 72 x 0.1 ml Tubes	Aluminum block for manual reaction setup with a single-channel pipet in 72 x 0.1 ml tubes	9018901
Loading Block 96 x 0.2 ml Tubes	Aluminum block for manual reaction set-up in a standard 8 x 12 array using 96 x 0.2 ml tubes	9018905

Product	Contents	Cat. no.
Strip Tubes and Caps, 0.1 ml (250)	250 strips of 4 tubes and caps for 1000 reactions	981103
Strip Tubes and Caps, 0.1 ml (2500)	10 x 250 strips of 4 tubes and caps for 10,000 reactions	981106
PCR Tubes, 0.2 ml (1000)	1000 thin-walled tubes for 1000 reactions	981005
PCR Tubes, 0.2 ml (10000)	10 x 1000 thin-walled tubes for 1000 reactions	981008

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