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artus[®] HCV RG RT-PCR Kit Handbook

 24 (catalog no. 4518263)

 96 (catalog no. 4518265)

Version 1

IVD

Quantitative in vitro diagnostics

For use with Rotor-Gene[®] Q Instruments



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Kit Contents

artus HCV RG RT-PCR Kit			(24)	(96)
Catalog no.			4518263	4518265
Number of reactions			24	96
Blue	Hep. C Virus RG Master A		2 x 12 reactions	8 x 12 reactions
Violet	Hep. C Virus RG Master B		2 x 12 reactions	8 x 12 reactions
Red	Hep. C Virus RG QS 1* (10 ⁴ IU/μl)	QS	200 μl	200 μl
Red	Hep. C Virus RG QS 2* (10 ³ IU/μl)	QS	200 μl	200 μl
Red	Hep. C Virus RG QS 3* (10 ² IU/μl)	QS	200 μl	200 μl
Red	Hep. C Virus RG QS 4* (10 ¹ IU/μl)	QS	200 μl	200 μl
Green	Hep. C Virus RG IC [†]	IC	1000 μl	2 x 1000 μl
White	Water (PCR grade)		1000 μl	1000 μl
	Handbook		1	1

* Quantitation standard.

† Internal control.

Symbols



Contains reagents sufficient for <N> tests



Use by



In vitro diagnostic medical device



Catalog number



Lot number



Material number

	Components
	Contains
	Number
	Global Trade Item Number
	Temperature limitation
	Manufacturer
	Consult instructions for use
	Important note

Storage

The components of the *artus* HCV RG RT-PCR Kit should be stored at -30°C to -15°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 $2-8^{\circ}\text{C}$ should not exceed a period of 5 hours.

Intended Use

The *artus* HCV RG RT-PCR Kit is an in vitro nucleic acid amplification test for the quantitation of hepatitis C virus (HCV) RNA in human plasma. This diagnostic test kit utilizes the reverse transcription polymerase chain reaction (RT-PCR) and is configured for use with Rotor-Gene Q Instruments. The test can quantitate HCV RNA over the range of $65 - 1 \times 10^6$ HCV IU/ml.

 The *artus* HCV RG RT-PCR Kit may not be used with Rotor-Gene Q 2plex Instruments.

The *artus* HCV RG RT-PCR Kit is intended for use in conjunction with clinical presentation and other laboratory markers for disease prognosis and for use as an aid in assessing viral response to antiretroviral treatment as measured by changes in EDTA plasma HCV RNA levels. The *artus* HCV RG RT-PCR Kit is not

intended to be used as a screening test for HCV or as a diagnostic test to confirm the presence of HCV infection.

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.

Quality Control

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

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.

Introduction

The *artus* HCV RG RT-PCR Kit constitutes a ready-to-use system for the detection of HCV RNA using polymerase chain reaction (PCR) on Rotor-Gene Q Instruments. The Hep. C Virus RG Master A and B contain reagents and enzymes for the reverse transcription and specific amplification of a 240 bp region of the HCV genome, and for the direct detection of the specific amplicon in fluorescence channel Cycling Green of the Rotor-Gene Q MDx, Rotor-Gene Q, or Rotor-Gene 6000, or Cycling A.FAM™ (source 470 nm, detector 510 nm) of the Rotor-Gene 3000.

In addition, the *artus* HCV RG RT-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 Orange of the Rotor-Gene Q MDx, Rotor-Gene Q, or Rotor-Gene 6000, or A.ROX™ (source 585 nm, detector 610 nm) of the Rotor-Gene 3000. The detection limit of the analytical HCV RT-PCR (see “Analytical sensitivity”, page 8) is not reduced. External positive controls (Hep. C Virus RG QS 1–4) are supplied, which allow the determination of the amount of viral RNA. For further information, see “Quantitation”, page 21.

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 C is a liver inflammation caused by the virus of the same name. As opposed to the other hepatitis viruses A, B, D, or E, infection with the hepatitis C virus (HCV) leads, in a high number of cases, to chronic liver disease. An infection with HCV often produces no symptoms for a relatively long period of time. For this reason, most patients are not aware of their HCV infection. Therapy, however, is most effective in the earliest stages of the disease. Currently, interferon α (in combination with Ribavirin) is the only proven, effective treatment. However, it is also known that only some chronic hepatitis C patients respond to interferon therapy. Hence, under certain circumstances, this expensive patient treatment can be unfavorable and may

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

have serious side effects such as a debilitation of the immune system, leading to exacerbations (e.g., lip herpes, shingles).

Performance characteristics

Analytical sensitivity

To determine the analytical sensitivity of the *artus* HCV RG RT-PCR Kit, a standard dilution series was set up from 10 IU/ μ l to nominal 0.0316 IU/ μ l in vitro transcribed RNA copies and analyzed with the *artus* HCV RG RT-PCR Kit. 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* HCV RG RT-PCR Kit is 0.19 IU/ μ l ($p = 0.05$). This means that there is a 95% probability that 0.19 IU/ μ l will be detected.

The analytical sensitivity in consideration of the purification (QIAamp[®] DSP Virus Kit) of the *artus* HCV RG RT-PCR Kit on Rotor-Gene Instruments was determined using a dilution series of the WHO International HCV RNA Standard from 500 to nominal 5 HCV IU/ml spiked in clinical plasma specimens. These were subjected to RNA extraction using the QIAamp DSP Virus Kit (extraction volume: 0.5 ml, elution volume: 25 μ l). Each of the 9 dilutions was analyzed with the *artus* HCV RG RT-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* HCV RG RT-PCR Kit in combination with Rotor-Gene Instruments is 33.6 IU/ml ($p = 0.05$). This means that there is a 95% probability that 33.6 IU/ml will be detected.

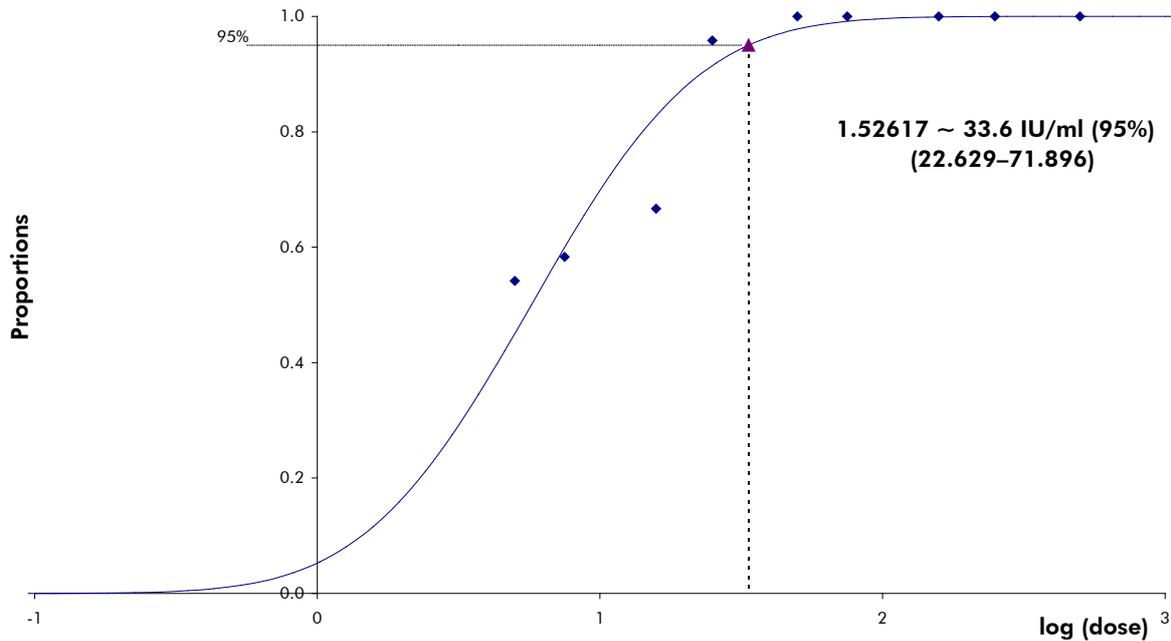


Figure 1. Probit analysis: HCV (Rotor-Gene 3000). Analytical sensitivity in consideration of the purification (QIAamp DSP Virus Kit, QIAGEN) of the *artus* HCV RG RT-PCR Kit on the Rotor-Gene 3000.

Specificity

The specificity of the *artus* HCV RG RT-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 subtypes and genotypes has thus been ensured.

Moreover, the specificity was validated with 100 different HCV negative plasma samples. These did not generate any signals with the HCV specific primers and probes, which are included in the Hep. C Virus RG Masters.

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

Table 1. Testing of the specificity of relevant genotypes

Virus	Genotype	Source	HCV (Cycling Green/ A.FAM)	Internal control (Cycling Orange/ A.ROX)
Hepatitis C virus	1	NIBSC, HemaCare, University of Essen	+	+
Hepatitis C virus	2	NIBSC, HemaCare, University of Essen	+	+
Hepatitis C virus	3	NIBSC, HemaCare, University of Essen	+	+
Hepatitis C virus	4	NIBSC, HemaCare, University of Essen	+	+
Hepatitis C virus	5	NIBSC, HemaCare, University of Essen	+	+
Hepatitis C virus	6	NIBSC, HemaCare, University of Essen	+	+

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

Control group	HCV (Cycling Green/ A.FAM)	Internal control (Cycling Orange/ A.ROX)
Human immunodeficiency virus 1	-	+
Hepatitis A virus	-	+
Hepatitis B virus	-	+
Human herpesvirus 1 (herpes simplex virus 1)	-	+
Human herpesvirus 2 (herpes simplex virus 2)	-	+
Human herpesvirus 3 (varicella-zoster virus)	-	+
Human herpesvirus 5 (cytomegalovirus)	-	+

Table continued on next page

Table 2. Continued

Control group	HCV (Cycling Green/ A.FAM)	Internal control (Cycling Orange/ A.ROX)
Human T cell leukemia virus type 1 and type 2	–	+
Human herpesvirus 6A	–	+
Human herpesvirus 6B	–	+
Human herpesvirus 8 (Kaposi's sarcoma herpesvirus)	–	+
Enterovirus	–	+
Parvovirus B19	–	+
Dengue fever	–	+
Yellow fever	–	+
<i>Aspergillus flavus</i>	–	+
<i>Aspergillus fumigatus</i>	–	+
<i>Candida albicans</i>	–	+
<i>Chlamydia trachomatis</i>	–	+
<i>Cryptosporidium parvum</i>	–	+
<i>Filobasidiella neoformans</i>	–	+
<i>Mycoplasma pneumoniae</i>	–	+
<i>Pneumocystis carinii</i>	–	+
<i>Staphylococcus</i> sp.	–	+
<i>Streptococcus agalactiae</i>	–	+
<i>Staphylococcus aureus</i>	–	+
<i>Streptococcus pyogenes</i>	–	+

Linear Range

The linear range (analytical measurement) of the *artus* HCV RG RT-PCR Kit was determined by analyzing a dilution series of an HCV in vitro transcript from

1 x 10⁷ IU/μl to 1 IU/μl. The dilution series has been calibrated against the WHO International HCV RNA Standard.

Each dilution was tested in replicates (n = 8) using the *artus* HCV RG RT-PCR Kit on the Rotor-Gene Instruments.

The linear range of the *artus* HCV RG RT-PCR Kit has been determined to cover concentrations from 1 IU/μl to at least 1 x 10⁷ IU/μl.

The linear range in consideration of the purification of the *artus* HCV RG RT-PCR Kit was determined by analyzing samples from Acrometrix. The purification was carried out in replicates (n = 6) from 50 IU/ml to 10³ IU/ml and in replicates (n = 4) from 5 x 10³ IU/ml to 10⁶ IU/ml using the QIAamp DSP Virus Kit (extraction volume: 0.5 ml, elution volume: 25 μl). Each of the samples was analyzed using the *artus* HCV RG RT-PCR Kit on Rotor-Gene Instruments. The linear range in consideration of the purification of the *artus* HCV RG RT-PCR Kit has been determined to cover concentrations from 65 IU/ml to at least 10⁶ IU/ml (see Figure 2).

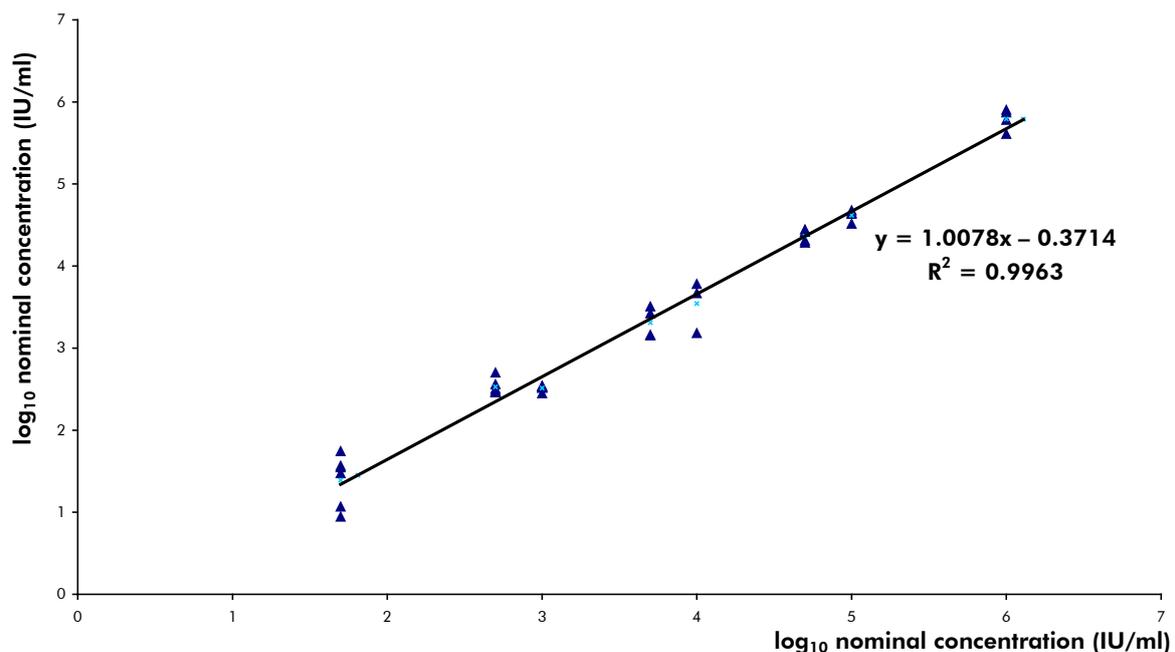


Figure 2. Linear Range of the *artus* HCV RG RT-PCR Kit. Calculation of the linear range in consideration of the purification. The straight line was determined by a linear regression of the log₁₀ calculated concentrations with the log₁₀ nominal concentrations. The equation of the regression line is included in the figure.

Precision

The precision data of the *artus* HCV RG RT-PCR Kit on Rotor-Gene Instruments allow the 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* HCV RG RT-PCR Kit have been collected using the quantitation standard of the lowest concentration (QS 4; 10 IU/ μ l). 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). In addition, precision data for quantitative results in IU/ μ l were determined using the corresponding C_T values (see Table 4). Based on these results, the overall statistical spread of any given sample with the mentioned concentration is 1.52% (C_T) or 25.71% (concentration), and 0.75% (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

	C_T value	Standard deviation	Coefficient of variation (%)
Intra-assay variability: Hep. C Virus RG QS 4	32.81	0.09	0.28
Intra-assay variability: Hep. C Virus RG IC	30.04	0.08	0.27
Inter-assay variability: Hep. C Virus RG QS 4	32.14	0.5	1.57
Inter-assay variability: Hep. C Virus RG IC	30.23	0.22	0.71
Inter-batch variability: Hep. C Virus RG QS 4	32.56	0.48	1.46
Inter-batch variability: Hep. C Virus RG IC	30.28	0.24	0.78
Total variance: Hep. C Virus RG QS 4	32.41	0.49	1.52
Total variance: Hep. C Virus RG IC	30.29	0.29	0.75

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

	Standard deviation	Variance	Coefficient of variation (%)
Intra-assay variability: Hep. C Virus RG QS 4	0.64	0.41	6.34
Inter-assay variability: Hep. C Virus RG QS 4	1.00	1.00	9.93
Inter-batch variability: Hep. C Virus RG QS 4	3.92	15.34	37.35
Total variance: Hep. C Virus RG QS 4	2.63	6.93	25.71

Robustness

The verification of the robustness allows the determination of the total failure rate of the *artus* HCV RG RT-PCR Kit. 100 HCV negative samples of plasma were spiked with 2 IU/ μ l elution volume of HCV control RNA (approximately threefold concentration of the analytical sensitivity limit). After extraction using the QIAamp DSP Virus Kit, these samples were analyzed with the *artus* HCV RG RT-PCR Kit. For all HCV samples the failure rate was 0%. In addition, the robustness of the internal control was assessed by purification and analysis of 100 HCV negative plasma samples. The total failure rate was 0%. Inhibitions were not observed. Thus, the robustness of the *artus* HCV RG RT-PCR Kit is $\geq 99\%$.

Reproducibility

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

Diagnostic evaluation

The *artus* HCV RG RT-PCR Kit was evaluated in a study. Comparing the *artus* HCV RG RT-PCR Kit to the COBAS[®] TaqMan[®] HCV Test, 276 plasma specimens were analyzed retrospectively. All plasma specimens had previously been analyzed positive or negative using the COBAS TaqMan HCV Test for routine diagnostics.

HCV RNA for testing the *artus* HCV RG RT-PCR Kit was isolated using the QIAamp DSP Virus Kit, and analysis was carried out on the Rotor-Gene 6000 Instrument. For comparative testing with the COBAS TaqMan HCV Test, HCV

RNA was analyzed according to the instructions of the manufacturer provided in the package insert. The results obtained by using the *artus* HCV RG PCR Kit were compared to those of the COBAS TaqMan HCV Test (see Table 5 and Figure 3).

137 of 139 samples that tested positive with the COBAS TaqMan HCV Test also tested positive with the *artus* HCV RG RT-PCR Kit. All of the 137 samples that tested negative with the COBAS TaqMan HCV Test also tested negative with the *artus* HCV RG RT-PCR Kit.

If the results of the COBAS TaqMan HCV Test are taken as reference, the diagnostic sensitivity is 100%, and the diagnostic specificity is 98.6%.

Table 5. Results of the 276 analyzed retrospective EDTA plasma samples

		COBAS TaqMan HCV Test		
		+	-	Total
<i>artus</i> HCV RG RT-PCR Kit	+	137	2	139
	-	0	137	137

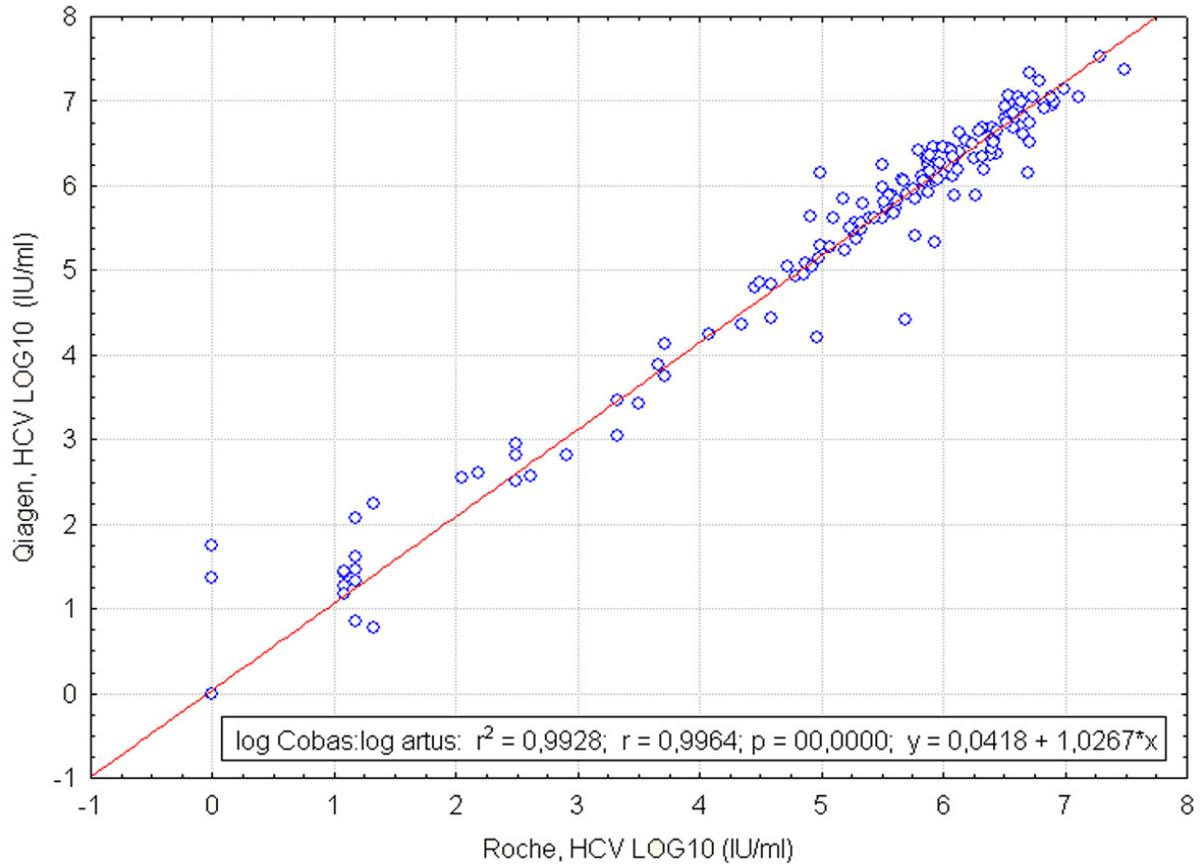


Figure 3. Comparison of the COBAS TaqMan HCV Test (Roche, HCV; with sample purification using the COBAS AmpliPrep system) with the *artus* HCV RG RT-PCR Kit (QIAGEN, HCV; with sample purification using the QIAamp DSP Virus Kit). Correlation of quantitative results from both test systems (Table 5) was 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.

- RNA isolation kit (see “RNA isolation”, page 20)
- Pipets (adjustable)*
- Sterile pipet tips with filters
- Vortex mixer*
- Benchtop centrifuge* with rotor for 2 ml reaction tubes
- Rotor-Gene Q MDx, Rotor-Gene Q, or Rotor-Gene Instrument*[†] with fluorescence channels for Cycling Green and Cycling Orange or with fluorescence channels for Cycling A.FAM and Cycling A.ROX
- Rotor-Gene Q MDx/Rotor-Gene Q software version 1.7.94 or higher (Rotor-Gene 6000 software version 1.7.65, 1.7.87, 1.7.94; Rotor-Gene 3000 software version 6.0.23)
- 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.

[†] The *artus* HCV RG RT-PCR Kit may not be used with Rotor-Gene Q 2plex Instruments.

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

i All samples have to be treated as potentially infectious material.

Only the following sample materials are permissible, for which the following rules and particular instructions regarding collection, transport, and storage have to be strictly observed.

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

The internal validation of the *artus* HCV RG RT-PCR Kit has been performed using human EDTA plasma samples. Other sample materials are not validated. Please use only the recommended RNA isolation kit (see “RNA isolation”, page 20) 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, too fine capillary needles should not be employed. 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 overhead directly after sample collection (8 x, do not agitate).

i Samples from heparinized humans must not be used (see “Interfering substances”, page 19).

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 has to 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 RNA 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. It is not recommended 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 the 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.

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

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

RNA isolation

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

i 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”).

i The internal control of the *artus* HCV RG RT-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 the assessment of the purification

Internal control

An internal control (Hep. C Virus RG IC) is supplied. This allows the user both to control the RNA 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 RNA is eluted in 60 μl Elution Buffer (AVE). Hence, 6 μl of the internal control should be added initially.

i The internal control and carrier RNA (see “RNA isolation”, above) 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).

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

The internal control can optionally be used exclusively to check for possible PCR inhibition. For this application, add the internal control directly to the mixture of Hep. C Virus RG Master A and Hep. C Virus RG Master B, as described in step 2b of the protocol (page 23).

Quantitation

The enclosed quantitation standards (Hep. C Virus RG QS 1–4) are treated as previously purified samples and the same volume is used (20 μ l). 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 as standards with the specified concentrations (see the instrument user manual).

i The quantitation standards are defined as IU/ μ l.* The following equation has to be applied to convert the values determined using the standard curve into IU/ml of sample material:

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

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 HCV standard (WHO).

Protocol: PCR and Data Analysis

Important points before starting

- Before beginning the procedure, read “Important Notes”, pages 18–21.
- Take time to familiarize yourself with the Rotor-Gene Q Instrument 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 4 quantitation standards supplied (Hep. C Virus RG QS 1–4) 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 RNA 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 20). In this case, prepare a master mix according to Table 6.**

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

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

Number of samples	1	12
Hep. C Virus RG Master A	12 μ l	144 μ l
Hep. C Virus RG Master B	18 μ l	216 μ l
Hep. C Virus RG IC	0 μ l	0 μ l
Total volume	30 μl	360 μl

- 2b. The internal control must be added directly to the mixture of Hep. C Virus Master A and Hep. C Virus Master B. 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
Hep. C Virus RG Master A	12 μ l	144 μ l
Hep. C Virus RG Master B	18 μ l	216 μ l
Hep. C Virus RG IC	2 μ l	24 μ l
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 μ l of the master mix into each PCR tube. Then add 20 μ l of the eluted sample RNA (see Table 8). Correspondingly, 20 μ l of at least one of the quantitation standards (Hep. C Virus RG QS 1–4) must be used as a positive control and 20 μ l of water (Water, PCR grade) as a negative control.**

Table 8. Preparation of PCR assay

Number of samples	1	12
Master mix	30 μ l	30 μ l each
Sample	20 μ l	20 μ l 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 HCV RNA, create a temperature profile according to the following steps.**

Setting the general assay parameters	Figures 4, 5, 6
Reverse transcription of the RNA	Figure 7
Initial activation of the hot-start enzyme	Figure 8
Amplification of the cDNA	Figure 9
Adjusting the fluorescence channel sensitivity	Figure 10
Starting the run	Figure 11

All specifications refer to the Rotor-Gene Q MDx/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”.

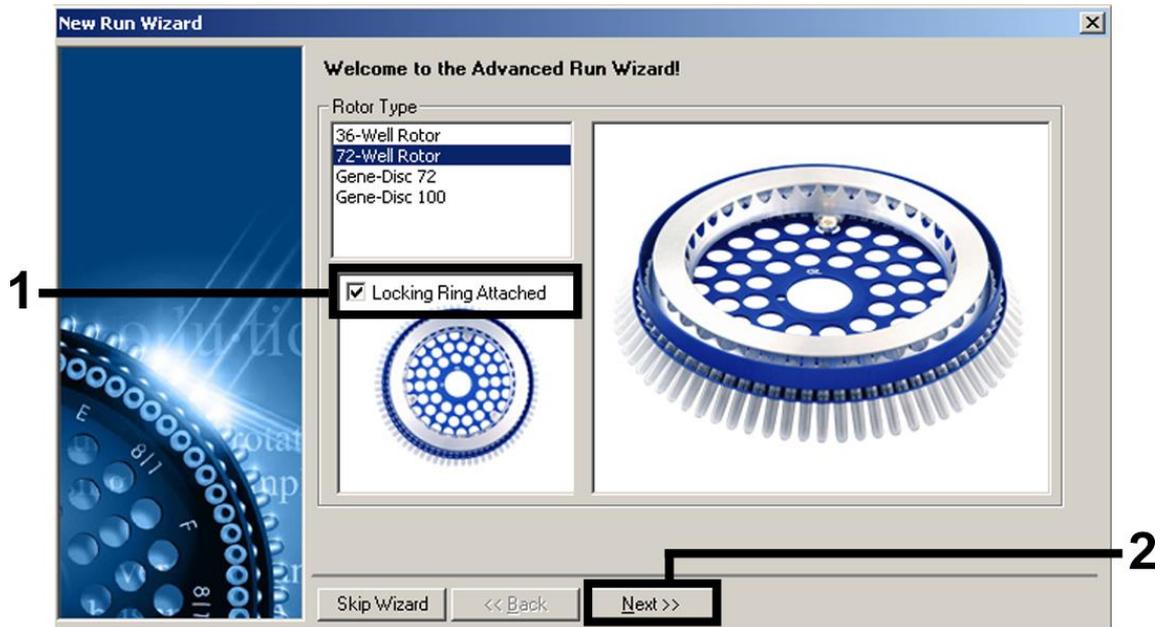


Figure 4. The “New Run Wizard” dialog box.

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

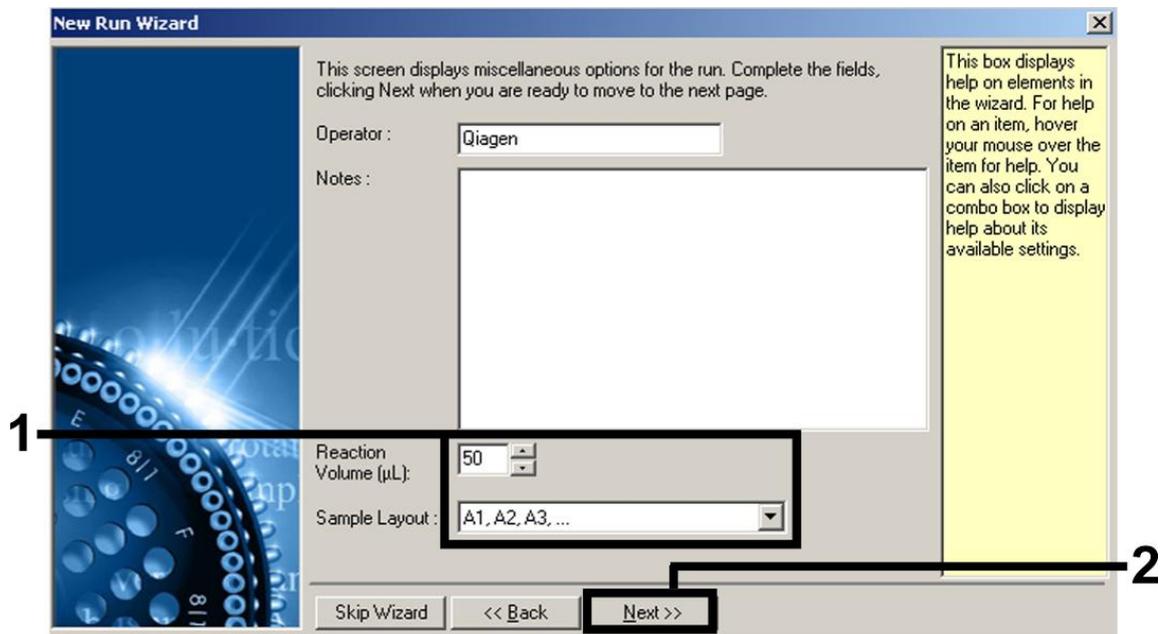


Figure 5. Setting the general assay parameters.

- Click the "Edit Profile" button in the next "New Run Wizard" dialog box (Figure 6), and program the temperature profile as shown in Figures 6–9).

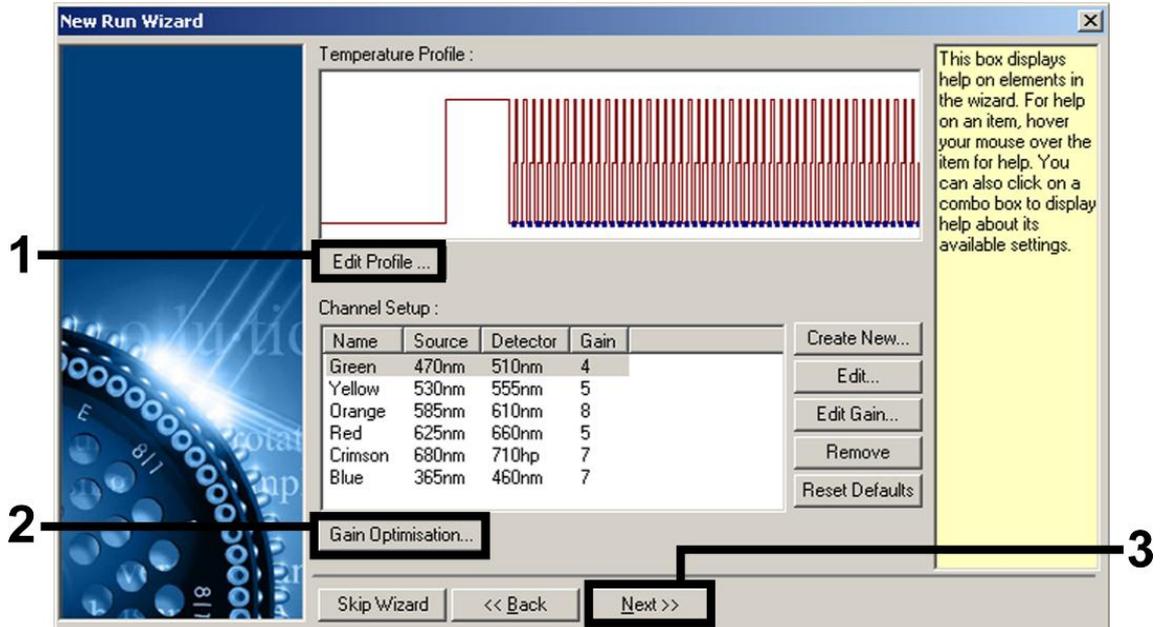


Figure 6. Editing the profile.

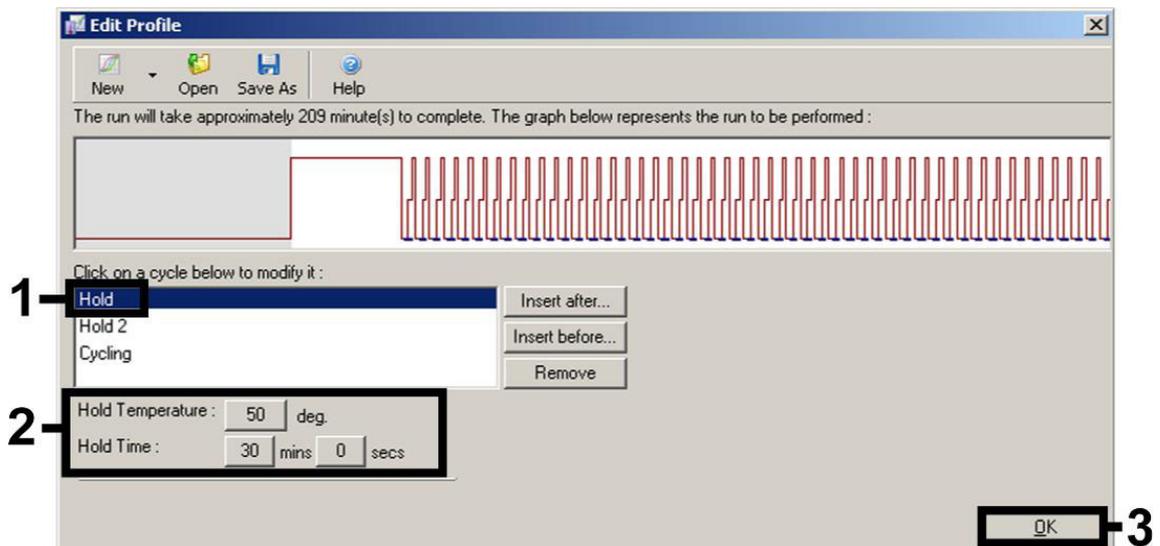


Figure 7. Reverse transcription of the RNA.

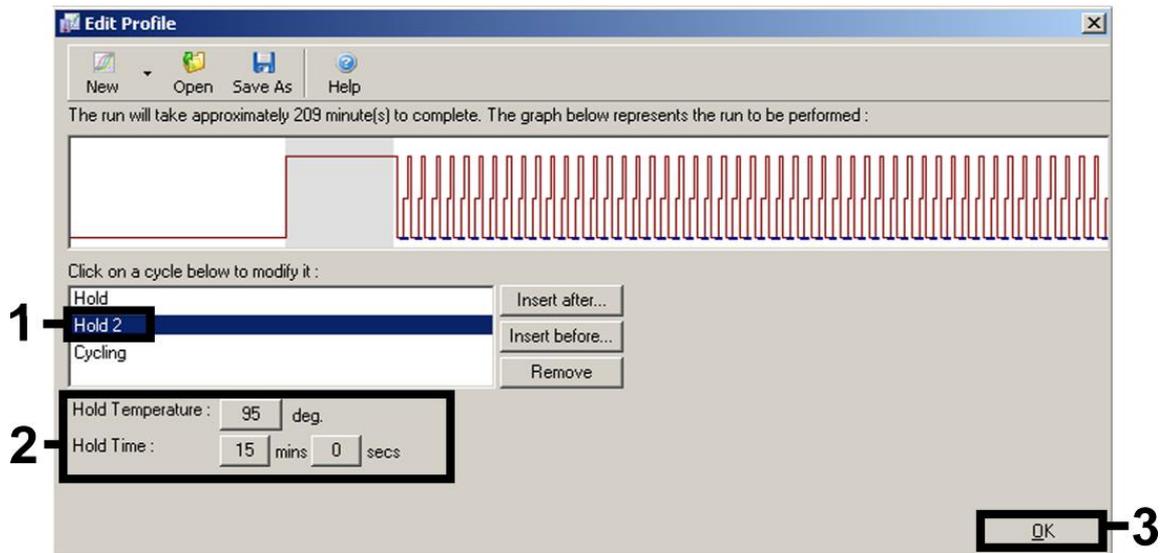


Figure 8. Initial activation of the hot-start enzyme.

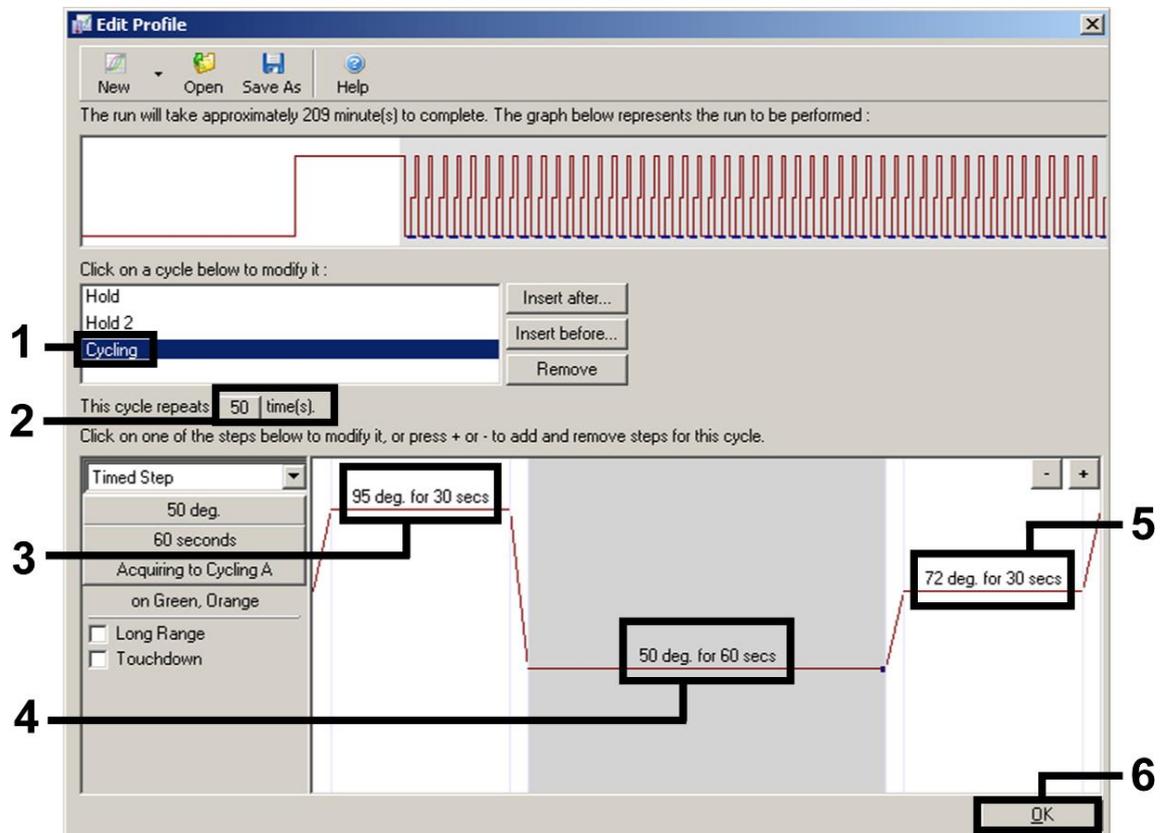


Figure 9. Amplification of the cDNA. Note that, on the Rotor-Gene 3000, the software will define the fluorescence dyes as "FAM/Sybr, ROX".

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 50 to match the annealing temperature of the amplification program (Figure 10).

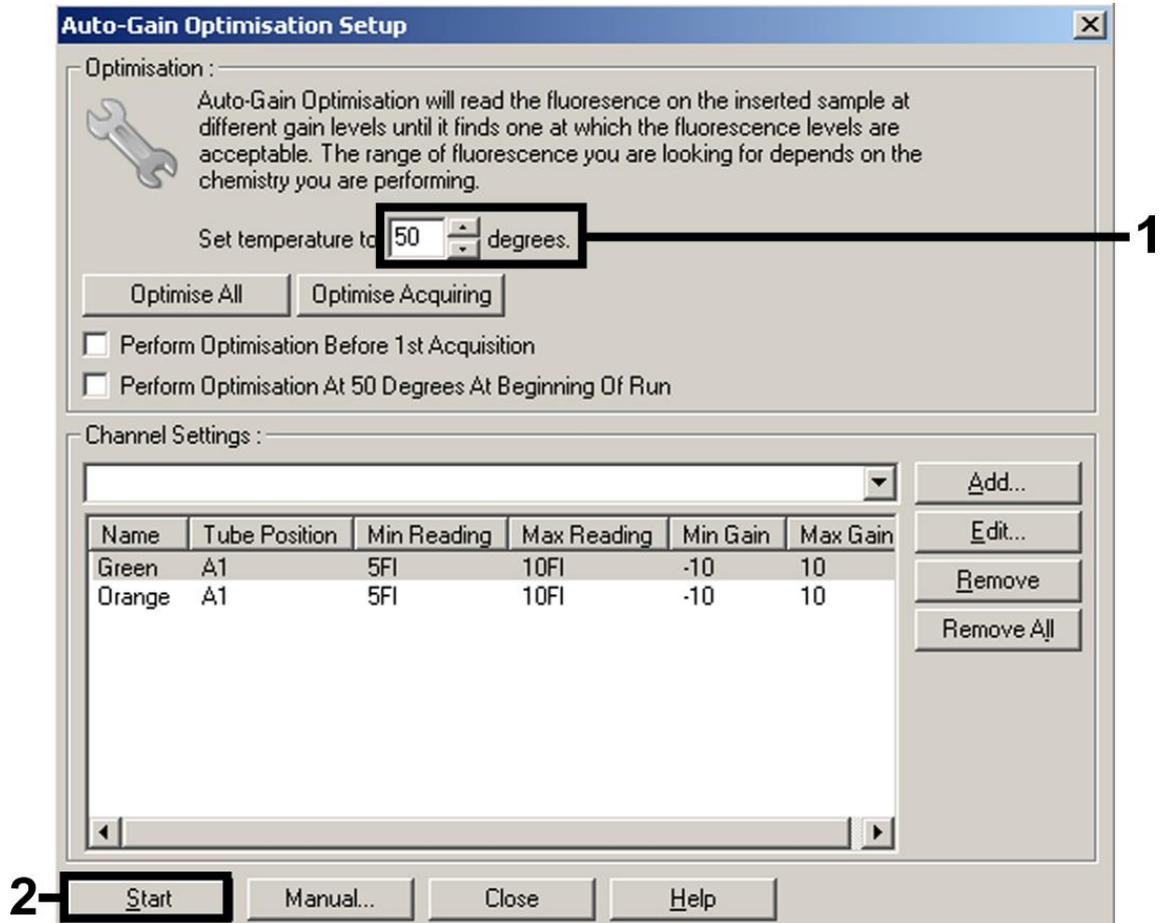


Figure 10. Adjusting the fluorescence channel sensitivity. Note that, on the Rotor-Gene 3000, the software will define the fluorescence dyes as "FAM/Sybr" and "ROX".

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 11). Click “Start Run”.

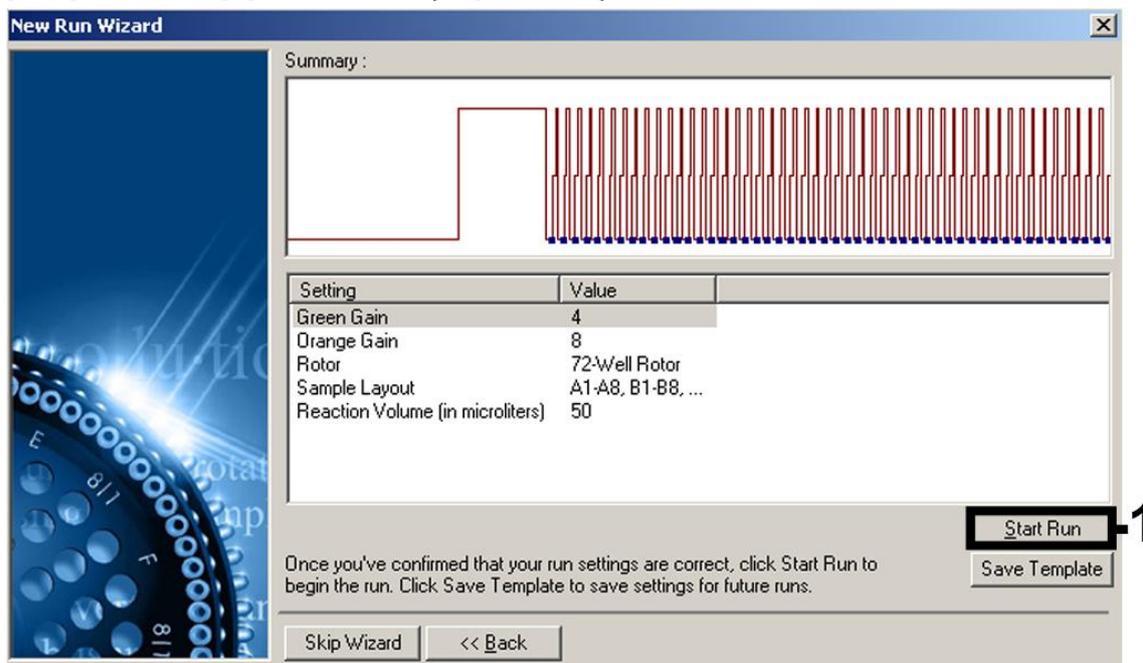


Figure 11. Starting the run. Note that, on the Rotor-Gene 3000, the software will define the fluorescence dyes as “FAM/Sybr” and “ROX”.

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 12 and Figure 13.

Table 9 shows guidelines for interpretation of quantitative results.

- 11a. A signal is detected in fluorescence channel Cycling Green. The result of the analysis is positive: the sample contains HCV RNA.**

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

i Note that, on the Rotor-Gene 3000, the relevant channels are Cycling A.FAM for the positive signal and Cycling A.ROX 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 Orange channel. In the sample no HCV RNA is detectable. It can be considered negative.**

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

i Note that, on the Rotor-Gene 3000, the relevant channels are Cycling A.ROX 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 Orange channels.

No result can be concluded.

Information regarding error sources and their solution can be found in “Troubleshooting Guide”, page 32.

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

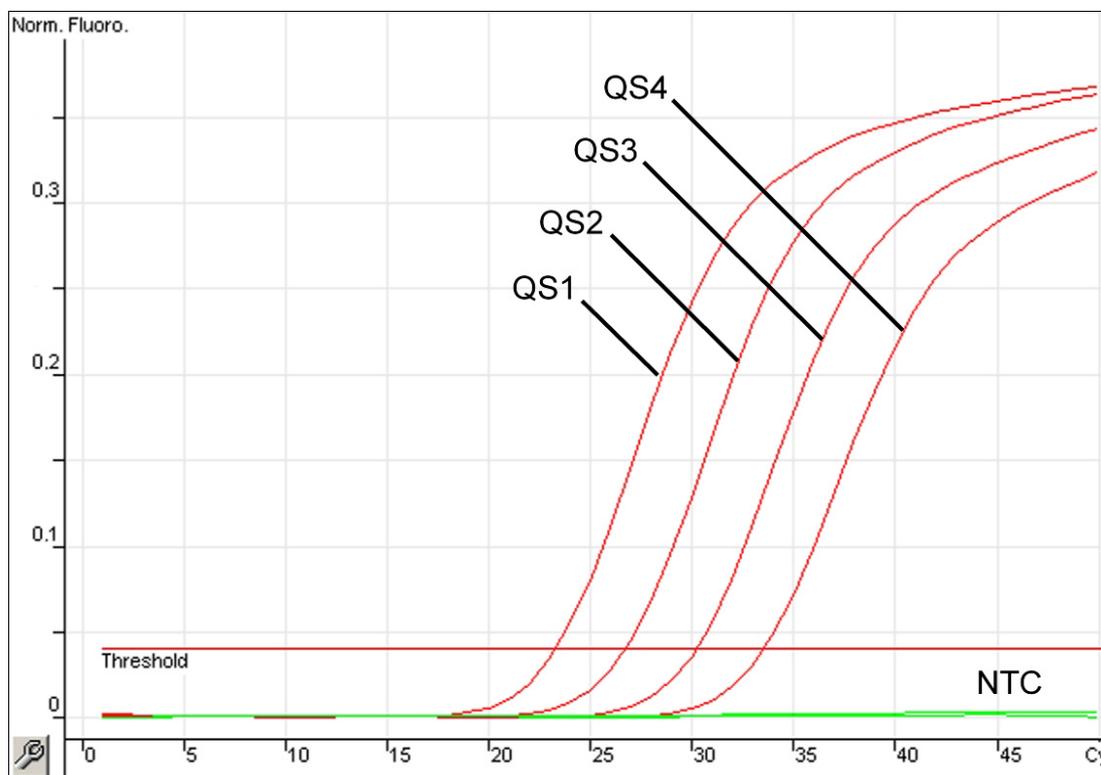


Figure 12. Detection of the quantitation standards (Hep. C Virus RG QS 1–4) in fluorescence channel Cycling Green. NTC: No template control (negative control).

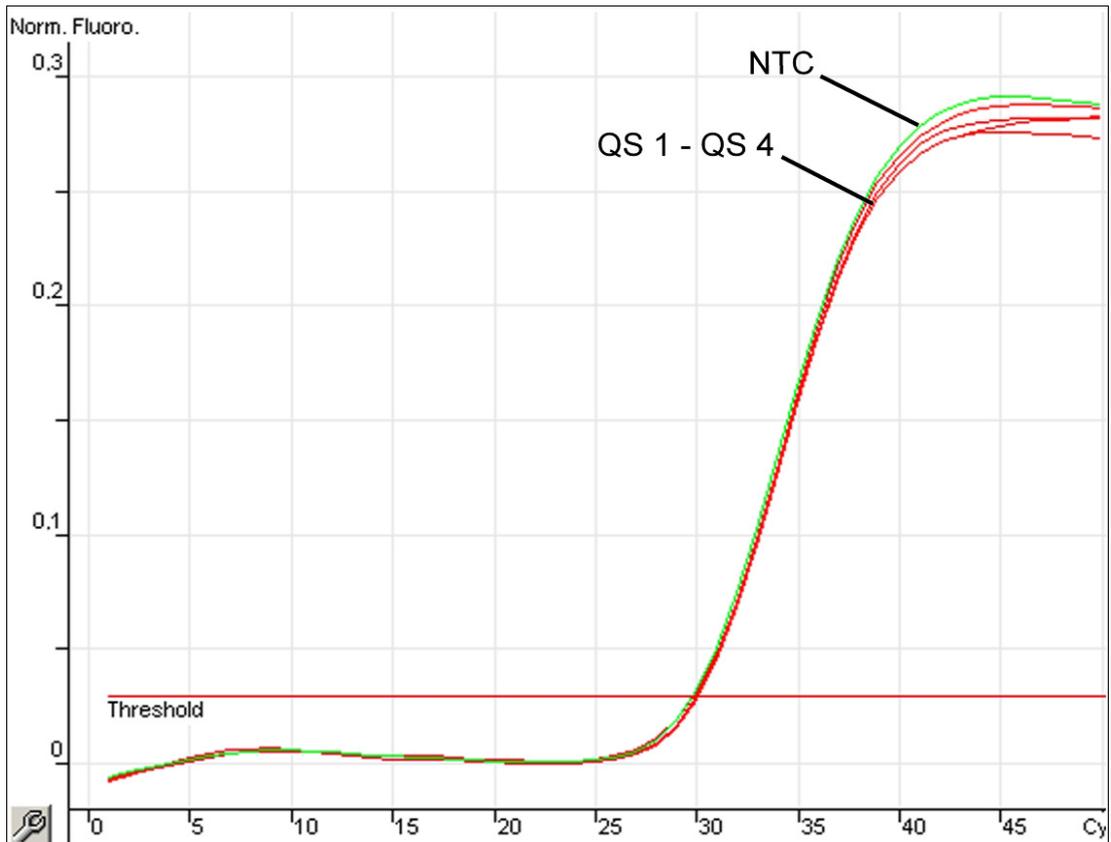


Figure 13. Detection of the internal control (IC) in fluorescence channel Cycling Orange with simultaneous amplification of the quantitation standards (Hep. C Virus RG QS 1–4). NTC: No template control (negative control).

Table 9. Interpretation of quantitative results

Result	Interpretation
HCV RNA >34 IU/ml	The result is within the determined test range. The detection probability of HCV RNA is >95%. The positive test result is statistically ensured.
HCV RNA <34 IU/ml	The result is outside the determined test range. The reproducibility of the positive result is not assured.
HCV RNA negative	No HCV RNA was detected.

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 (Hep. C Virus RG QS 1–4) 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 HCV RT-PCR and the fluorescence channel Cycling Orange or Cycling A.ROX for the internal control RT-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 22. |
| 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 22. |
| 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 <i>artus</i> HCV RG RT-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 in fluorescence channel Cycling Orange or Cycling A.ROX 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.
- ⓘ Make sure that during the RNA isolation the recommended additional centrifugation step has been carried out before the elution in order to remove any residual ethanol (see "RNA isolation", page 20).
- c) RNA 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 RNA during the extraction. Make sure that you use the recommended isolation method (see "RNA isolation", page 20) 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* HCV RG RT-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) A contamination occurred during preparation of the PCR
- ① Repeat the PCR with new reagents in replicates.
 - ① If possible, close the PCR tubes directly after addition of the sample to be tested.
 - ① Make sure to pipet the positive controls last.
 - ① Make sure that work space and instruments are decontaminated at regular intervals.
- b) A contamination occurred during extraction
- ① Repeat the extraction and PCR of the sample to be tested using new reagents.
 - ① 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.
<i>artus</i> HCV RG RT-PCR Kit (24)	For 24 reactions: 2 Masters, 4 Quantitation Standards, Internal Control, Water (PCR grade)	4518263
<i>artus</i> HCV RG RT-PCR Kit (96)	For 96 reactions: 2 Masters, 4 Quantitation Standards, Internal Control, Water (PCR grade)	4518265
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 and accessories		
Rotor-Gene Q MDx 5plex Platform	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training not included	9002022
Rotor-Gene Q MDx 5plex System	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training	9002023
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: includes 1-year warranty on parts and labor, installation and training not included	9002032

Product	Contents	Cat. no.
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: includes 1-year warranty on parts and labor, installation and training	9002033
Rotor-Gene Q MDx 6plex Platform	Real-time PCR instrument with 6 channels (blue, green, yellow, orange, red, crimson), including laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training not included	9002042
Rotor-Gene Q MDx 6plex System	Real-time PCR instrument with 6 channels (blue, green, yellow, orange, red, crimson), including laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training	9002043
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
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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