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artus® CMV RG PCR Kit Handbook

Σ 24 (catalog no. 4503263)

Σ 96 (catalog no. 4503265)

Version 1

IVD

Quantitative in vitro diagnostics

For use with Rotor-Gene® Q Instruments

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QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, GERMANY

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Contents

Intended Use

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

Summary and Explanation

The *artus* CMV RG PCR Kit constitutes a ready-to-use system for the detection of CMV DNA using polymerase chain reaction (PCR) on Rotor-Gene Q Instruments. The CMV RG Master contains reagents and enzymes for the specific amplification of a 105 bp region of the CMV 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™ of the Rotor-Gene 3000.

In addition, the *artus* CMV 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 MDx, Rotor-Gene Q, or Rotor-Gene 6000, or Cycling A.JOE™ of the Rotor-Gene 3000. The detection limit of the analytical CMV PCR (see "Analytical sensitivity", page 25) is not reduced. External positive controls (CMV QS 1–4) are supplied which allow the determination of the amount of viral DNA. For further information, see "Quantitation", page 19.

Pathogen information

The human cytomegalovirus (CMV) is found in infected persons in blood, tissues, and nearly all secretory fluids. Transmission can be oral, sexual, via blood transfusion or organ transplantation, intrauterine, or perinatal. Infection with CMV frequently leads to an asymptomatic infection followed by a lifelong persistence of the virus in the body. If symptoms occur, in teenagers or in adults, they resemble those of mononucleosis with fever, weak hepatitis, and general indisposition. Severe courses of CMV infection have been observed especially in those infected intrauterine and in immunodeficient patients.

Principle of the Procedure

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.*

Materials Provided

Kit contents

***artus* CMV RG PCR Kit**			**(24)**	**(96)**
Catalog no.			**4503263**	**4503265**
Number of reactions			**24**	**96**
Blue	CMV RG Master		2 x 12 reactions	8 x 12 reactions
Yellow	CMV Mg-Sol†	**Mg-Sol**	600 µl	600 µl
Red	CMV QS 1‡ (1 x 10^4 copies/µl)	**QS**	200 µl	200 µl
Red	CMV QS 2‡ (1 x 10^3 copies/µl)	**QS**	200 µl	200 µl
Red	CMV QS 3‡ (1 x 10^2 copies/µl)	**QS**	200 µl	200 µl
Red	CMV QS 4‡ (1 x 10^1 copies/µl)	**QS**	200 µl	200 µl
Green	CMV RG IC§	**IC**	1000 µl	2 x 1000 µl
White	Water (PCR grade)		1000 µl	1000 µl
	Handbook	HB	1	1

† Magnesium solution.

‡ Quantitation standard.

§ Internal control.

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

Materials Required but Not Provided

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.

Reagents

- DNA isolation kit (see "DNA isolation", page 10)

Consumables

- Sterile pipet tips with filters
- 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)

Equipment

- Pipets (adjustable)*
- 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 Yellow or with fluorescence channels for Cycling A.FAM and Cycling A.JOE
- 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)
- 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.

Warnings and Precautions

For in vitro diagnostic use

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.

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).

Reagent Storage and Handling

The components of the *artus* CMV 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.

Specimen Handling and Storage

Note: All samples have to be treated as potentially infectious material.

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

The validation of the *artus* CMV RG PCR Kit has been 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 10) 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 over-head directly after sample collection (8 x, do not agitate).

Note: Samples from heparinized humans must not be used (see "Interfering substances", page 9).

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.

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 (≤4.5 mg/dl) and lipids (≤1000 mg/dl) and hemolytic samples do not influence the system. Heparin 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.

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

Procedure

DNA isolation

The kits from QIAGEN shown in Table 1 are validated for viral DNA purification from the indicated human sample types for use with the *artus* CMV RG PCR Kit. Carry out the viral DNA purification according to the instructions in the kit handbooks.

Table 1. Purification kits validated for use with the *artus* CMV RG PCR Kit

Sample material	Sample size	Nucleic acid isolation kit	Catalog number (QIAGEN)	Carrier RNA
EDTA plasma	500 µl	QIAamp® DSP Virus Kit	60704	Included
EDTA plasma	400 µl	EZ1® DSP Virus Kit (48)*	62724	Included

* The EZ1 DSP Virus Kit is also available as CE-IVD-marked EASY*artus*® CMV RG PCR Kits, combined with the *artus* CMV RG PCR Kit (see page 34 for ordering information).

Note: 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 proceeding according to the information about the reconstitution and storage of the carrier RNA given in the instruction manual ("Preparing reagents and buffers").

Note: The internal control of the *artus* CMV RG PCR Kit can be used directly in the isolation procedure. Make sure to include one negative plasma sample in the isolation procedure. The corresponding signal of the internal control is the basis for the evaluation of the isolation (see "Internal control", below).

Internal control

An internal control (CMV RG 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.

Note: The internal control and carrier RNA (see "DNA isolation", page 10) 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).

Note: 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 $C_T = 27 \pm 3$ (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, 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 CMV RG Master and CMV Mg-Sol, as described in step 2b of the protocol (page 13).

Protocol: PCR and data analysis

Important points before starting

- 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 (CMV 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 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.**

   Note: It is highly recommended to add the internal control to CMV RG Master and CMV Mg-Sol used for the quantitation standards. For the quantitation standards, add the internal control directly to the CMV RG Master and CMV Mg-Sol, as described in step 2b of the protocol, and use this master mix for each quantitation standard (CMV QS 1–4).

2a. The internal control has already been added to the isolation (see "Internal control", page 10). In this case, prepare a master mix according to Table 2.

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

Table 2. Preparation of master mix (internal control used to monitor DNA isolation and check for PCR inhibition)

Number of samples	1	12
CMV RG Master	25 µl	300 µl
CMV Mg-Sol	5 µl	60 µl
CMV RG IC	0 µl	0 µl
Total volume	**30 µl**	**360 µl**

2b. The internal control must be added directly to the mixture of CMV RG Master and CMV Mg-Sol. In this case, prepare a master mix according to Table 3.

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

Table 3. Preparation of master mix (internal control used exclusively to check for PCR inhibition)

Number of samples	1	12
CMV RG Master	25 µl	300 µl
CMV Mg-Sol	5 µl	60 µl
CMV RG 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 4). Correspondingly, 20 µl of at least one of the quantitation standards (CMV QS 1–4) must be used as a positive control and 20 µl of water (Water, PCR grade) as a negative control.

Table 4. 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 CMV DNA, create a temperature profile according to the following steps.**

Setting the general assay parameters	**Figures 1, 2, 3**
Initial activation of the hot-start enzyme	**Figure 4**
Amplification of the DNA (touchdown PCR)	**Figure 5**
Adjusting the fluorescence channel sensitivity	**Figure 6**
Starting the run	**Figure 7**

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 1). Check the "Locking Ring Attached" box and click "Next".**

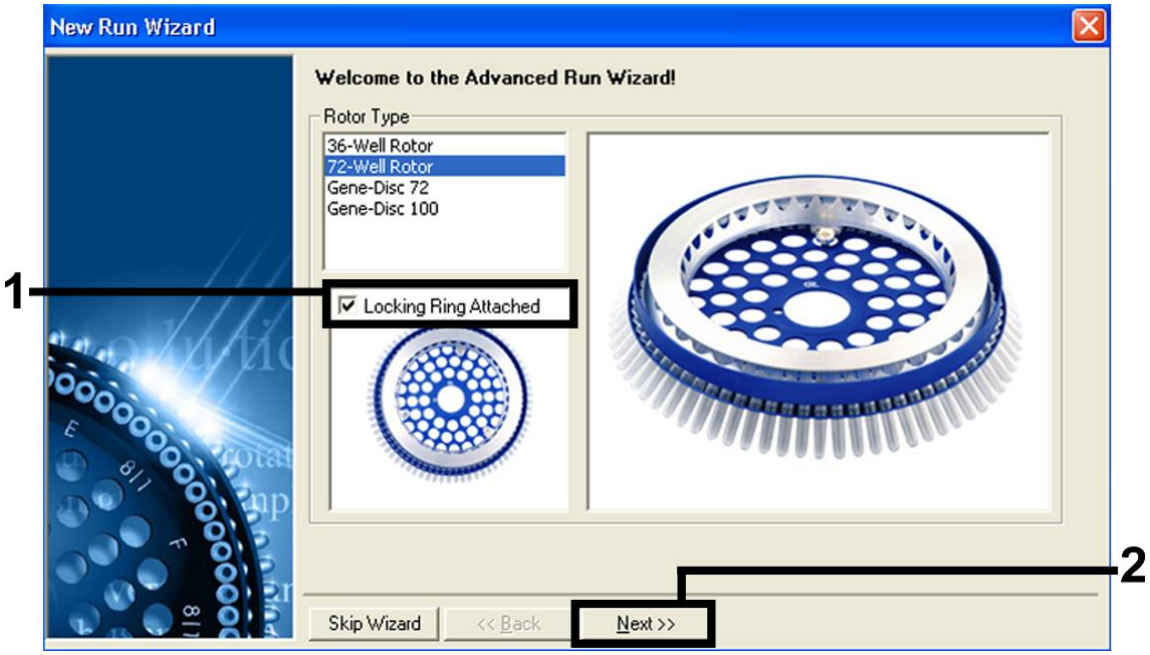

Figure 1. The "New Run Wizard" dialog box.

7. **Select *50* for the PCR reaction volume and click "Next" (Figure 2).**

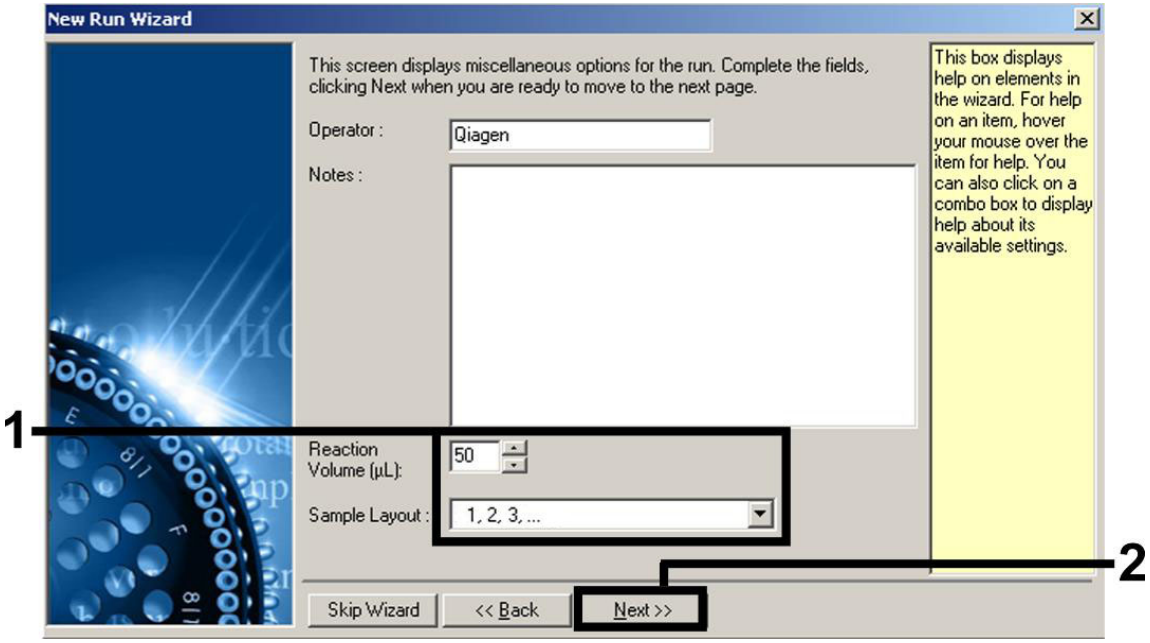

Figure 2. Setting the general assay parameters.

8. **Click the "Edit Profile" button in the next "New Run Wizard" dialog box (Figure 3), and program the temperature profile as shown in Figures 3–5).**

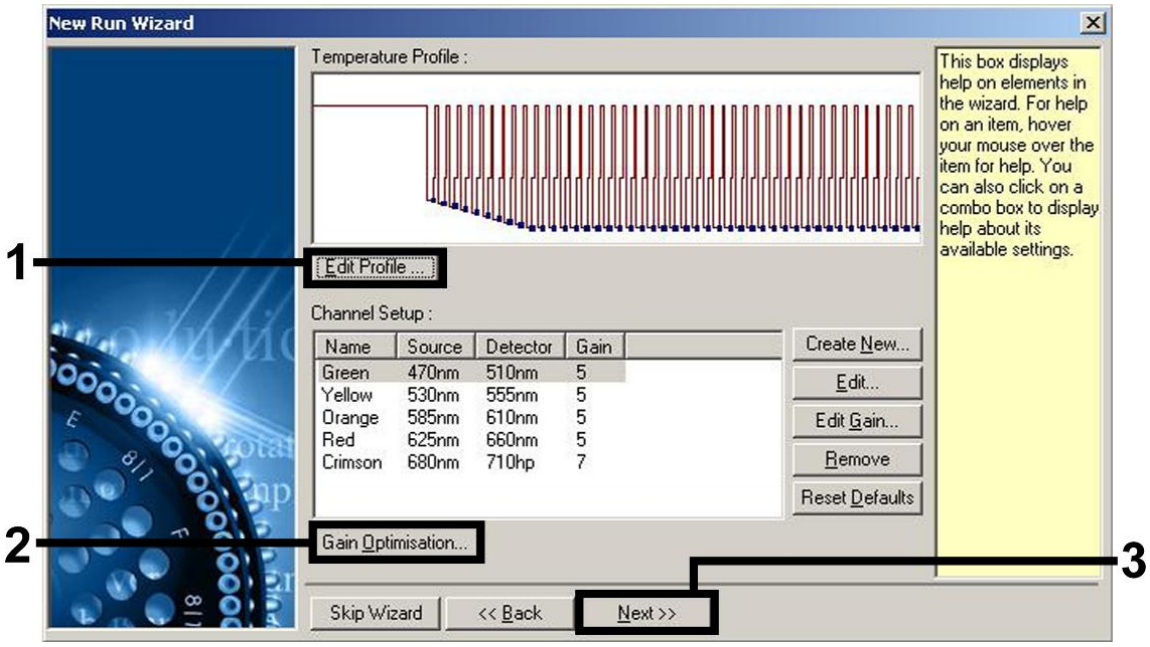

Figure 3. Editing the profile.

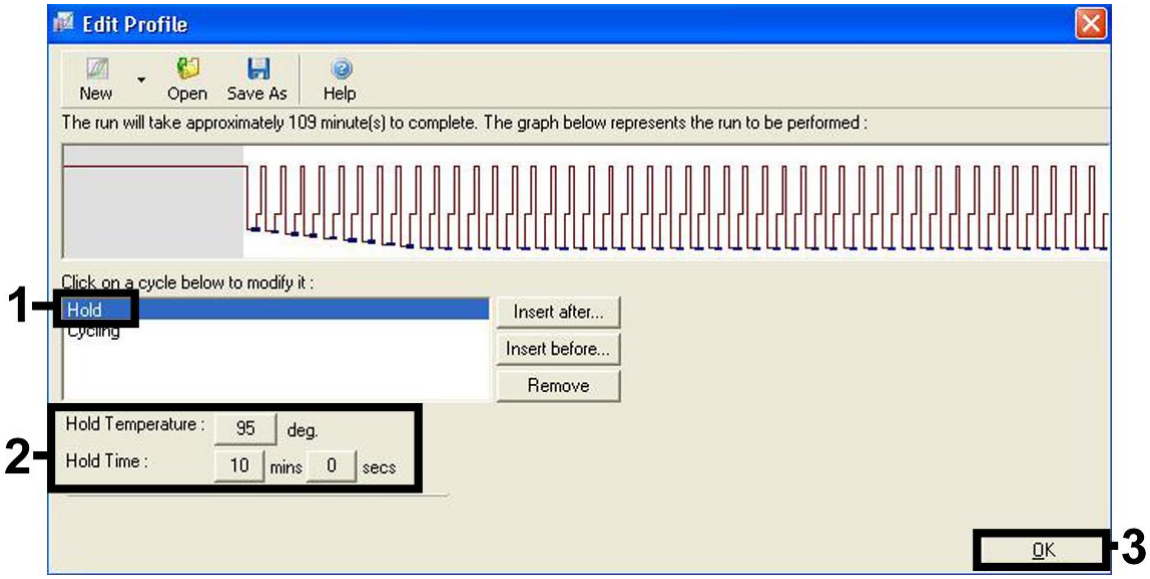

Figure 4. Initial activation of the hot-start enzyme.

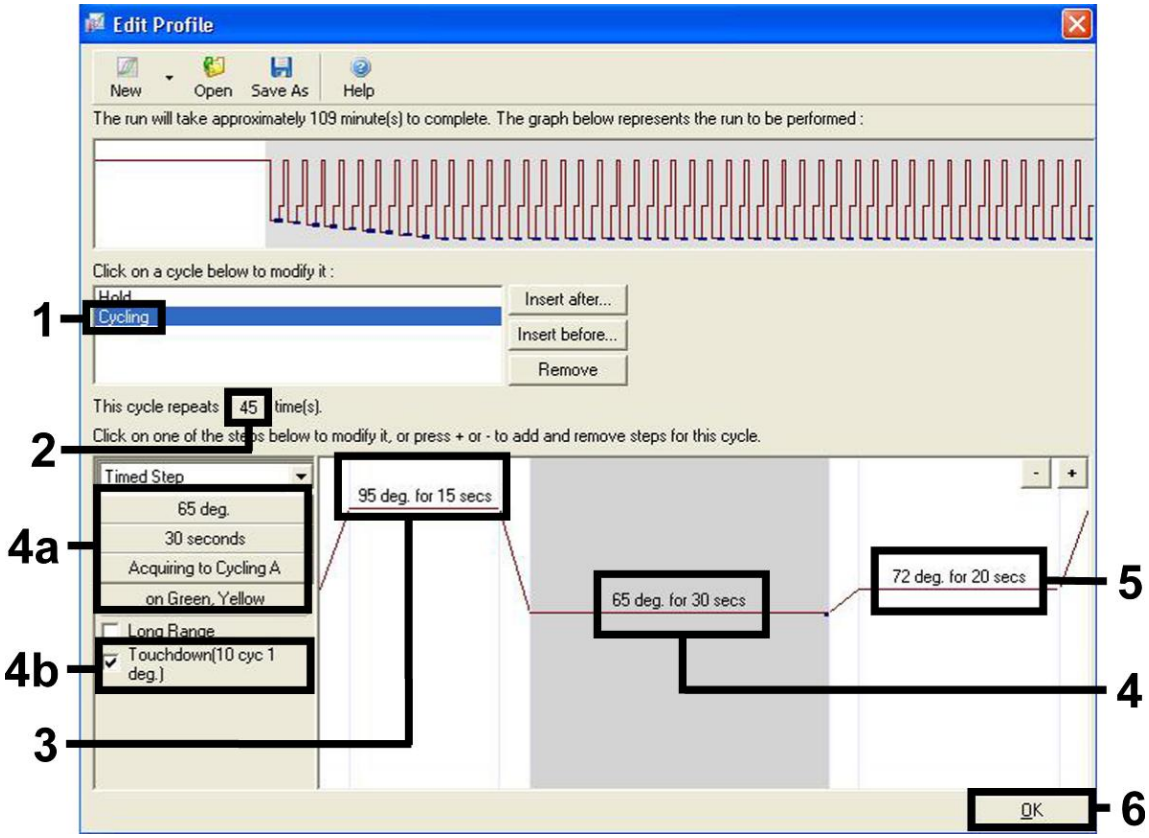

Figure 5. Amplification of the DNA. Make sure to activate the touchdown function for 10 cycles in the Annealing step. Note that, on the Rotor-Gene 3000, the software will define the fluorescence dyes as "FAM/Sybr, JOE".

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 3) to open the "Auto-Gain Optimisation Setup" dialog box. Set the calibration temperature to 65 to match the annealing temperature of the amplification program (Figure 6).**

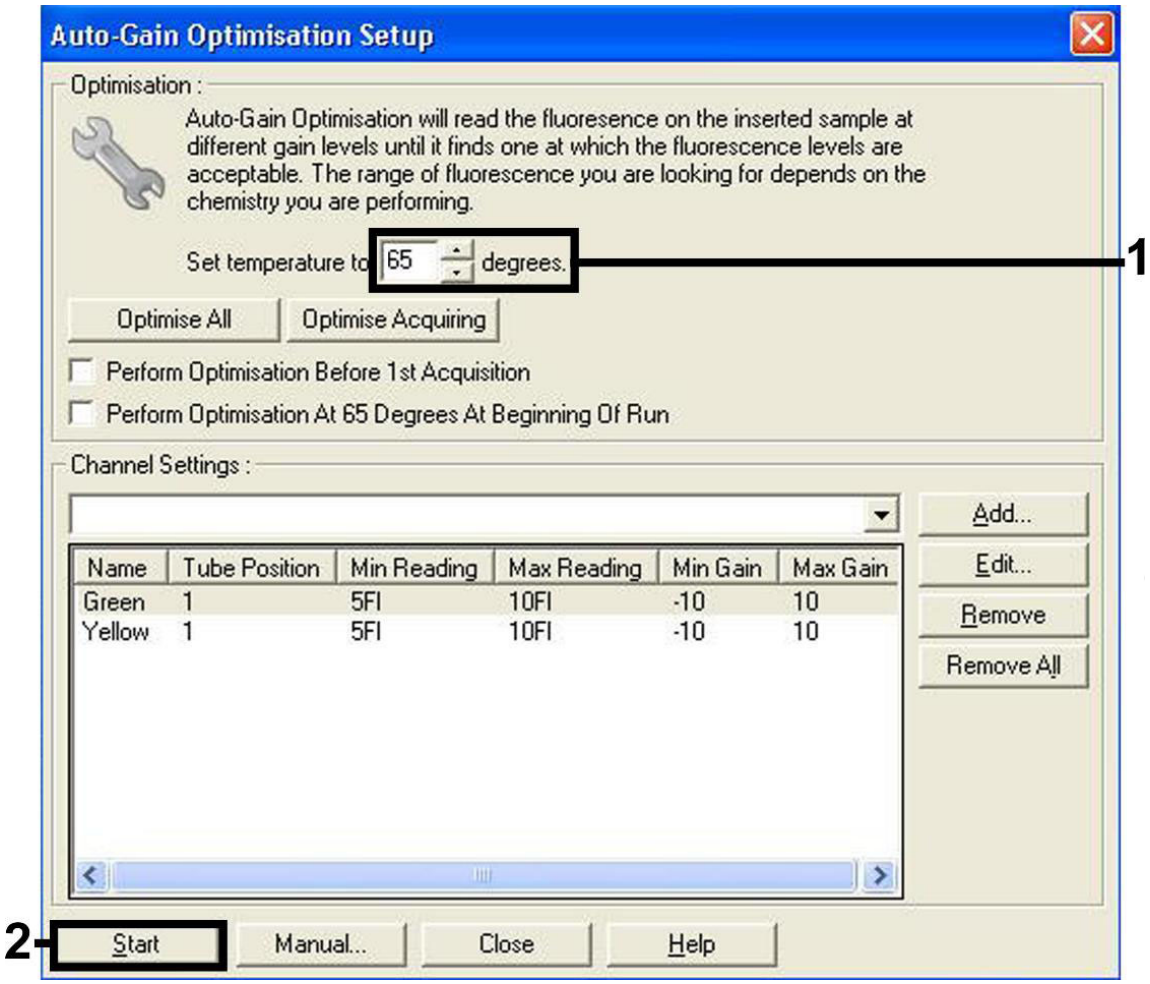

Figure 6. Adjusting the fluorescence channel sensitivity. Note that, on the Rotor-Gene 3000, the software will define the fluorescence dyes as "FAM/Sybr" and "JOE".

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 7). Click "Start Run".**

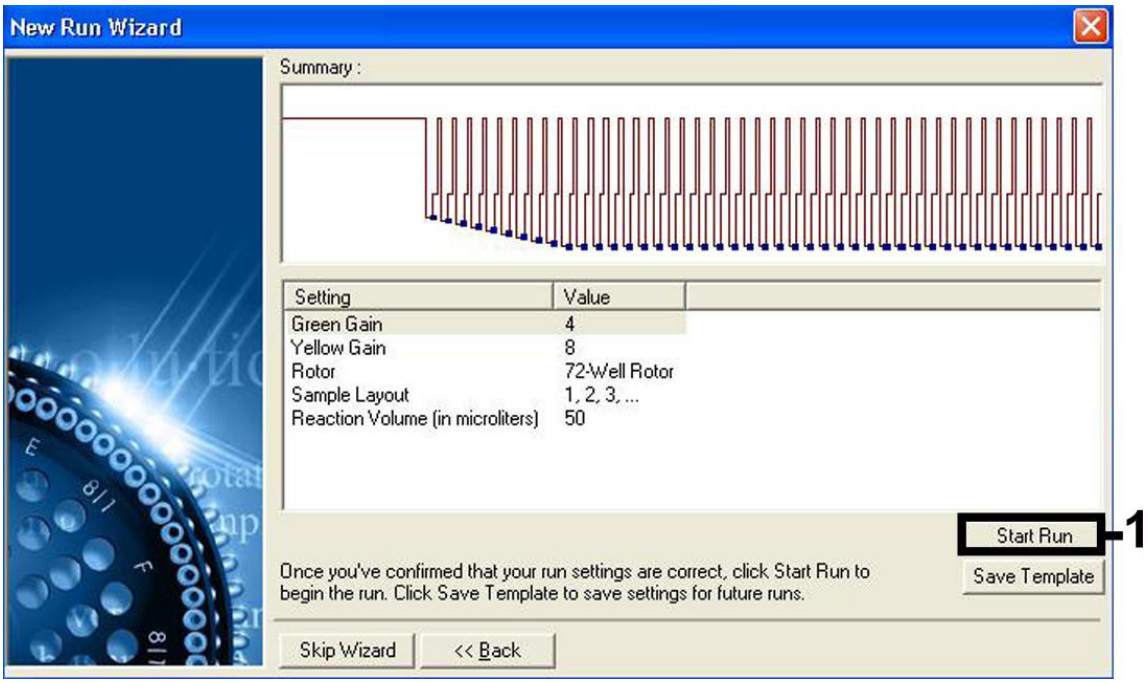

Figure 7. Starting the run. Note that, on the Rotor-Gene 3000, the software will define the fluorescence dyes as "FAM/Sybr" and "JOE".

Interpretation of Results

Quantitation

The enclosed quantitation standards (CMV QS 1–4) are treated as previously purified samples and the same volume is used (20 µl). To generate a standard curve on Rotor-Gene Q Instruments, all 4 quantitation standards should be used and defined in the "Edit Samples" dialog box as standards with the specified concentrations (see the instrument user manual).

Note: To ensure accurate quantitation, it is highly recommended to add the internal control to CMV RG Master and CMV Mg-Sol used for the quantitation standards. For this application, add the internal control directly to the CMV RG Master and CMV Mg-Sol, as described in step 2b of the protocol (page 13), and use this master mix for each quantitation standard (CMV QS 1–4).

Note: The quantitation standards are defined as copies/µl. The following equation has to be applied to convert the values determined using the standard curve into copies/ml of sample material:

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

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).

Results

Examples of positive and negative PCR reactions are given in Figure 8 and Figure 9.

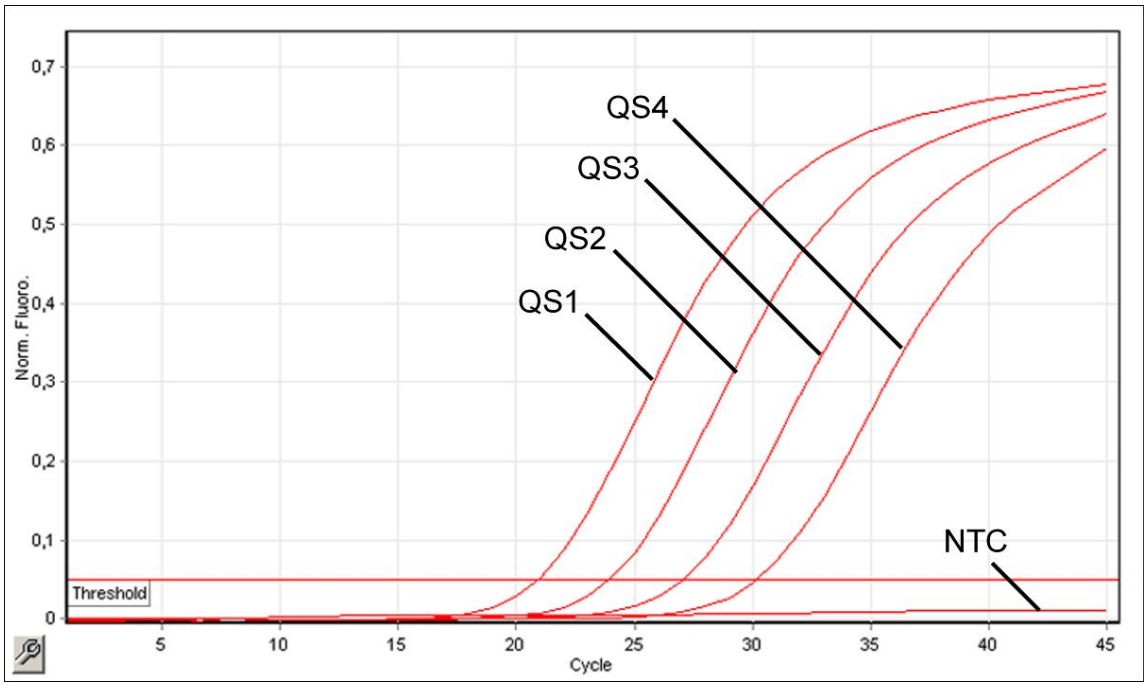

Figure 8. Detection of the quantitation standards (CMV QS 1–4) in fluorescence channel Cycling Green. NTC: No template control (negative control).

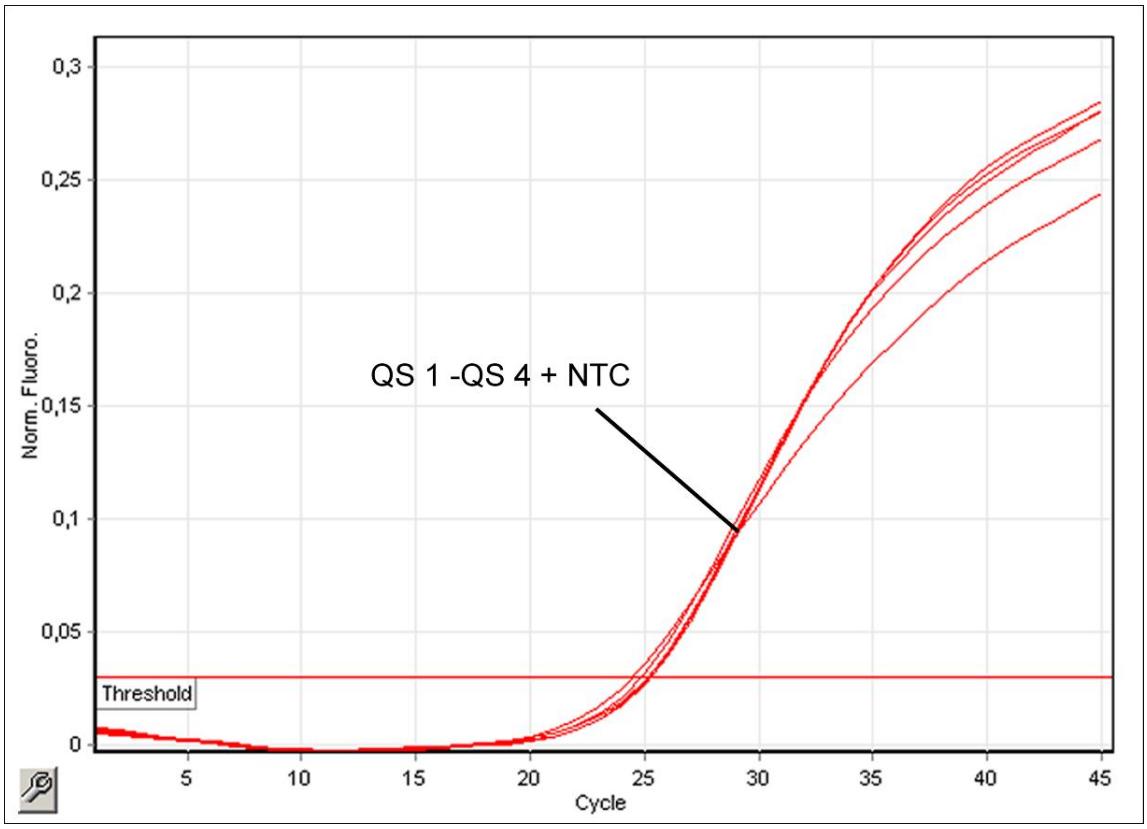

Figure 9. Detection of the internal control (IC) in fluorescence channel Cycling Yellow with simultaneous amplification of the quantitation standards (CMV QS 1–4). NTC: No template control (negative control).

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

In this case, the detection of a signal in the Cycling Yellow channel is dispensable, since high initial concentrations of CMV 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: On the Rotor-Gene 3000, the relevant channels are Cycling A.FAM for the positive signal and Cycling A.JOE for the internal control.

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 CMV DNA is detectable. It can be considered negative.

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

Note: 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.

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", below.

Note: On the Rotor-Gene 3000, the relevant channels are Cycling A.FAM and Cycling A.JOE.

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 (CMV 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 CMV 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 12.
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 12.
d) The storage conditions for one or more kit components did not comply with the instructions given in "Reagent Storage and Handling" (page 8)	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
e) The *artus* CMV 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.

Weak or no signal of the internal control of a negative plasma sample subjected to purification using the QIAamp DSP Virus Kit (C_T = 27 ± 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 10) 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 "Reagent Storage and Handling" (page 8)	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* CMV 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.

Quality Control

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

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.

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* CMV RG PCR Kit. The analytical detection limit in consideration of the purification is determined using CMV positive clinical specimens in combination with a particular extraction method. In contrast, the analytical detection limit is determined independent from the selected extraction method, using CMV DNA of known concentration.

To determine the analytical sensitivity of the *artus* CMV RG PCR Kit, a dilution series of CMV genomic DNA was set up from 10 to nominal 0.00316 copies/µl and analyzed on Rotor-Gene Instruments in combination with the *artus* CMV RG PCR Kit. Testing was carried out on 3 different days on 8 replicates. The results were determined by a probit analysis. A graphical illustration of the probit analysis on the Rotor-Gene 6000 is shown in Figure 10. The analytical detection limit of the *artus* CMV RG PCR Kit in combination with the Rotor-Gene Q MDx/Q/6000 and the Rotor-Gene 3000 is 0.36 copies/µl (p = 0.05) and 0.24 copies/µl (p = 0.05), respectively. This means that there is a 95% probability that 0.36 copies/µl or 0.24 copies/µl will be detected.

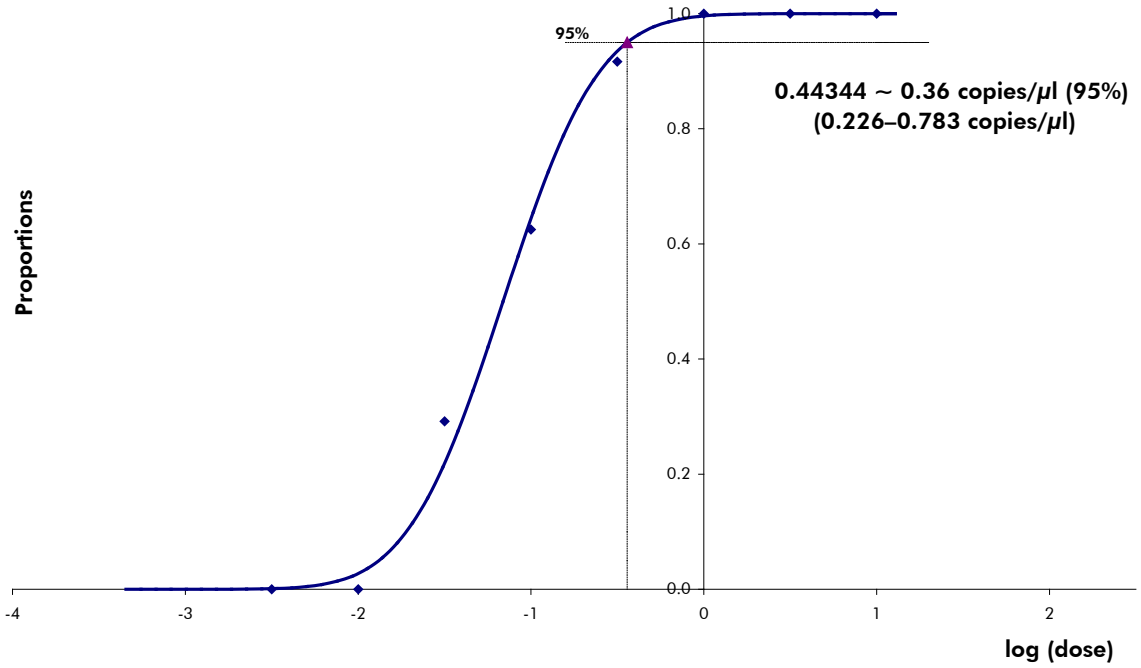

Figure 10. Probit analysis: CMV (Rotor-Gene 6000). Analytical sensitivity of the *artus* CMV RG PCR Kit on the Rotor-Gene 6000.

The analytical sensitivity in consideration of the purification (QIAamp DSP Virus Kit) of the *artus* CMV RG PCR Kit on Rotor-Gene Instruments was determined

using a dilution series of CMV virus material from 1000 to nominal 0.316 CMV copies/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: 60 µl). Each of the 8 dilutions was analyzed with the *artus* CMV 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 11. The analytical detection limit in consideration of the purification of the *artus* CMV RG PCR Kit in combination with the Rotor-Gene 3000 is 57.1 copies/ml (p = 0.05). This means that there is a 95% probability that 57.1 copies/ml will be detected.

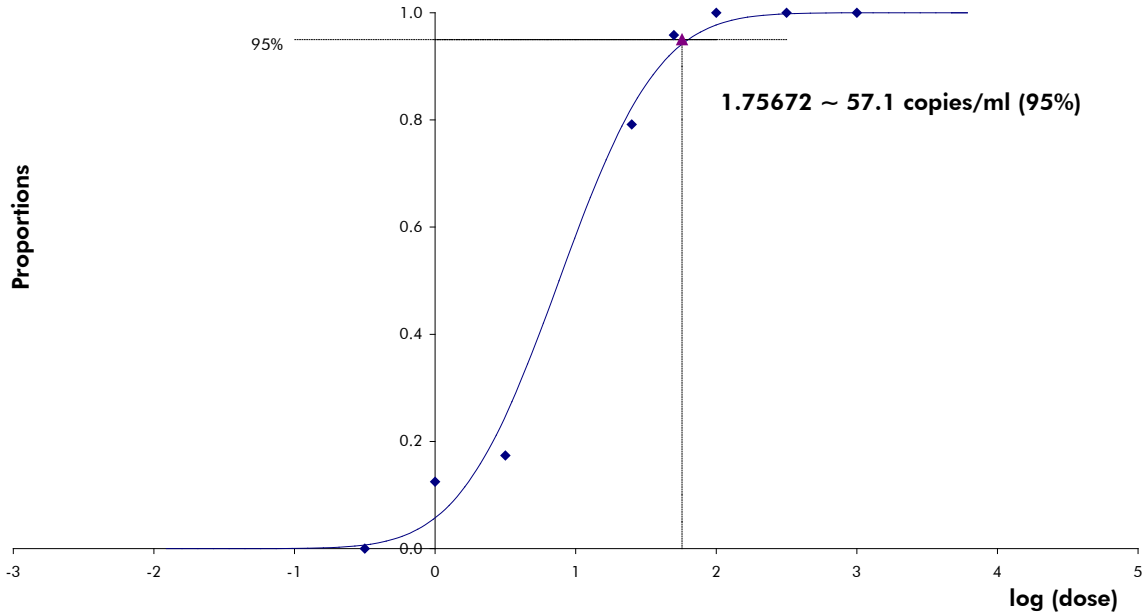

Figure 11. Probit analysis: CMV (Rotor-Gene 3000). Analytical sensitivity in consideration of the purification (QIAamp DSP Virus Kit, QIAGEN) of the *artus* CMV RG PCR Kit on the Rotor-Gene 3000.

The analytical sensitivity in consideration of the purification with EZ1 DSP Virus Kit (extraction volume: 0.4 ml, elution volume: 60 µl) using the EZ1 Advanced XL instrument of the *artus* CMV RG PCR Kit on the Rotor-Gene 6000 is 68.75 copies/ml (p = 0.05). This means that there is a 95% probability that 68.75 copies/ml will be detected.

Specificity

The specificity of the *artus* CMV 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 strains has thus been ensured.

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

A potential cross-reactivity of the *artus* CMV RG PCR Kit was tested using the control group listed in Table 5 (page 28). None of the tested pathogens has been reactive. No cross-reactivities appeared with mixed infections.

Table 5. Testing the specificity of the kit with potentially cross-reactive pathogens

Control group	CMV (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 6A	–	+
Human herpesvirus 6B	–	+
Human herpesvirus 7	–	+
Human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus)	–	+
Hepatitis A virus	–	+
Hepatitis B virus	–	+
Hepatitis C virus	–	+
Human immunodeficiency virus 1	–	+
Human T cell leukemia virus 1	–	+
Human T cell leukemia virus 2	–	+
West Nile virus	–	+
Enterovirus	–	+
Parvovirus B19	–	+

Precision

The precision data of the *artus* CMV RG PCR Kit have been collected by means of Rotor-Gene Instruments and 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* CMV RG PCR were collected using the quantitation standard of the lowest concentration (QS 4; 10 copies/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 6, page 30). In addition, precision data for quantitative results in copies/µl were determined using the corresponding C_T values (see Table 7, page 30). Based on these results, the overall statistical spread of any given sample with the mentioned concentration is 1.21% (C_T) or 14.38% (concentration), and 1.93% (C_T) for the detection of the internal control. These values are based on the totality of all single values of the determined variabilities.

Robustness

The verification of the robustness allows the determination of the total failure rate of the *artus* CMV RG PCR Kit. 100 CMV negative samples of plasma were spiked with CMV at a final concentration of 170 copies/ml (approximately threefold concentration of the analytical sensitivity limit). After extraction using the QIAamp DSP Virus Kit, these samples were analyzed with the *artus* CMV RG PCR Kit. For all CMV samples the failure rate was 0%. In addition, the robustness of the internal control was assessed by purification and analysis of 100 CMV negative plasma samples. Thus, the robustness of the *artus* CMV RG PCR Kit is ≥99%.

Reproducibility

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

Table 6. Precision data on basis of the C_T values

	Standard deviation	Variance	Coefficient of variation (%)
Intra-assay variability: CMV QS 4	0.17	0.03	0.57
Intra-assay variability: Internal control	0.31	0.10	1.16
Inter-assay variability: CMV QS 4	0.38	0.14	1.27
Inter-assay variability: Internal control	0.47	0.22	1.77
Inter-batch variability: CMV QS 4	0.33	0.11	1.10
Inter-batch variability: Internal control	0.53	0.28	2.02
Total variance: CMV QS 4	0.36	0.13	1.21
Total variance: Internal control	0.51	0.26	1.93

Table 7. Precision data on basis of the quantitative results (in copies/µl)

	Standard deviation	Variance	Coefficient of variation (%)
Intra-assay variability: CMV QS 4	1.34	1.80	13.30
Inter-assay variability: CMV QS 4	1.54	2.38	15.25
Inter-batch variability: CMV QS 4	1.46	2.12	14.41
Total variance: CMV QS 4	1.45	2.11	14.38

Diagnostic evaluation

The *artus* CMV RG PCR Kit was evaluated in a study. Comparing the *artus* CMV RG PCR Kit to the COBAS® AMPLICOR® CMV MONITOR® Test, 156 clinical EDTA plasma specimens were analyzed retrospectively and prospectively. All specimens had previously been analyzed positive or negative using the COBAS AMPLICOR CMV MONITOR for routine diagnostics.

CMV DNA for testing the *artus* CMV RG PCR Kit was isolated using the QIAamp DSP Virus Kit, with the internal control of the *artus* CMV RG PCR Kit added to the isolation, and analysis was carried out on the Rotor-Gene 3000. The specimens for the COBAS AMPLICOR CMV MONITOR Test were processed and analyzed according to the instructions of the manufacturer provided in the package insert.

All 11 samples that tested positive with the COBAS AMPLICOR CMV MONITOR Test also tested positive with the *artus* CMV RG PCR Kit. 123 of 145 samples that tested negative with the COBAS AMPLICOR CMV MONITOR Test also tested negative with the *artus* CMV RG PCR Kit. 22 discordant results were obtained (Table 8).

Table 8. Results of the comparative validation study

		COBAS AMPLICOR CMV MONITOR Test		
		+	**–**	**Total**
***artus* CMV RG PCR Kit**	+	11	22	33
	–	0	123	123

If the results of the COBAS AMPLICOR CMV MONITOR Test are taken as reference, the diagnostic sensitivity of all samples of the *artus* CMV RG PCR Kit is 100%, and the diagnostic specificity is 84.8%.

Further testing of the 22 discordant samples confirmed the results of the *artus* PCR Kits. Therefore it can be assumed that the discrepancy is based on the higher sensitivity of the *artus* CMV RG PCR Kit.

References

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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

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Ordering Information

Product	Contents	Cat. no.
artus CMV RG PCR Kit (24)	For 24 reactions: Master, Magnesium Solution, 4 Quantitation Standards, Internal Control, Water (PCR grade)	4503263
artus CMV RG PCR Kit (96)	For 96 reactions: Master, Magnesium Solution, 4 Quantitation Standards, Internal Control, Water (PCR grade)	4503265
EZ1 DSP Virus Kit — for automated, simultaneous purification of viral DNA and RNA from 1–14 serum, plasma, or CSF samples		
EZ1 DSP Virus Kit (48)	For 48 viral nucleic acid preps: Prefilled Reagent Cartridges, Disposable Tip Holders, Disposable Filter-Tips, Sample Tubes, Elution Tubes, Buffers, Carrier RNA	62724
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
EASY*artus* CMV RG PCR Kits — for fully automated viral nucleic acid purification using the EZ1 DSP Virus Kit, combined with pathogen detection using *artus* CMV RG PCR Kits		
EASY*artus* CMV RG PCR Kit 1	1 x EZ1 DSP Virus Kit, 1 x *artus* CMV RG PCR Kit	EA10323
EASY*artus* CMV RG PCR Kit 2	1 x EZ1 DSP Virus Kit, 2 x *artus* CMV RG PCR Kit	EA10324
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

Product	Contents	Cat. no.
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
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 comptuer, 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
Rotor-Gene Q MDx 2plex Platform	Real-time PCR cycler with 2 channels (green, yellow), laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training not included	9002002

Product	Contents	Cat. no.
Rotor-Gene Q MDx 2plex System	Real-time PCR cycler with 2 channels (green, yellow), laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training	9002003
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: includes 1-year warranty on parts and labor, installation and training not included	9002012
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: includes 1-year warranty on parts and labor, installation and training	9002013
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 10,000 reactions	981008

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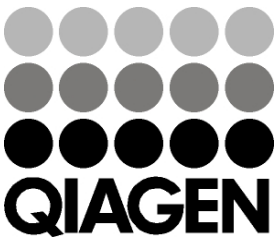

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