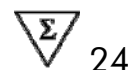


artus[®] CMV RG PCR Kit Handbook



For research use only. Not for use in diagnostic procedures.

For use with Rotor-Gene[®] Q Instruments



4503203



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QIAGEN GmbH, QIAGEN Strasse 1, D-40724 Hilden

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QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

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Kit Contents

artus CMV RG PCR Kit			(24)
Catalog no.			4503203
Number of reactions			24
Blue	CMV RG Master		2 x 12 reactions
Yellow	CMV Mg-Sol*	Mg-Sol	600 µl
Red	CMV QS 1 [†] (1 x 10 ⁴ copies/µl)	QS	200 µl
Red	CMV QS 2 [†] (1 x 10 ³ copies/µl)	QS	200 µl
Red	CMV QS 3 [†] (1 x 10 ² copies/µl)	QS	200 µl
Red	CMV QS 4 [†] (1 x 10 ¹ copies/µl)	QS	200 µl
Green	CMV IC [‡]	IC	1000 µl
White	Water (PCR grade)		1000 µl
	Handbook		1

* Magnesium solution.

[†] Quantitation standard.

[‡] Internal control.

Symbols



<N>

Contains sufficient for <N> tests



Use by



Catalog number



Lot number



Material number



Components



Contains



Number



Temperature limitation



Manufacturer



Consult instructions for use

Storage

The components of the *artus* CMV RG PCR Kit should be stored at -20°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.

Product Use Limitations

For research use only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

The product is to be used by personnel specially instructed and trained.

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.

Safety Information

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

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

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

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.

Introduction

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 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 or Rotor-Gene 6000, or Cycling A.JOE™ of the Rotor-Gene 3000. The detection limit of the analytical CMV PCR 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 16.

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.*

Specifications

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.

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

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/ μ l 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 1. The analytical detection limit of the *artus* CMV RG PCR Kit in combination with the Rotor-Gene Q/6000 and the Rotor-Gene 3000 is 0.36 copies/ μ l ($p = 0.05$) and 0.24 copies/ μ l ($p = 0.05$), respectively. This means that there is a 95% probability that 0.36 copies/ μ l or 0.24 copies/ μ l will be detected.

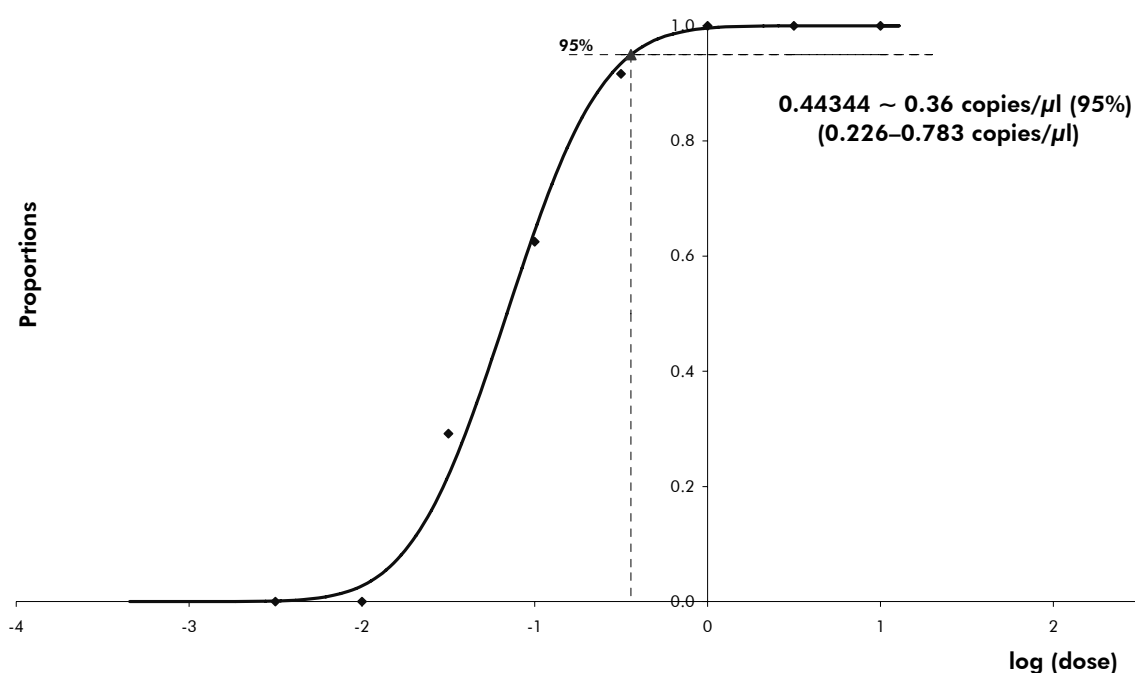


Figure 1. Probit analysis: CMV (Rotor-Gene 6000). Analytical sensitivity of the *artus* CMV RG PCR Kit on the Rotor-Gene 6000.

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 μ l). 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 2. 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.

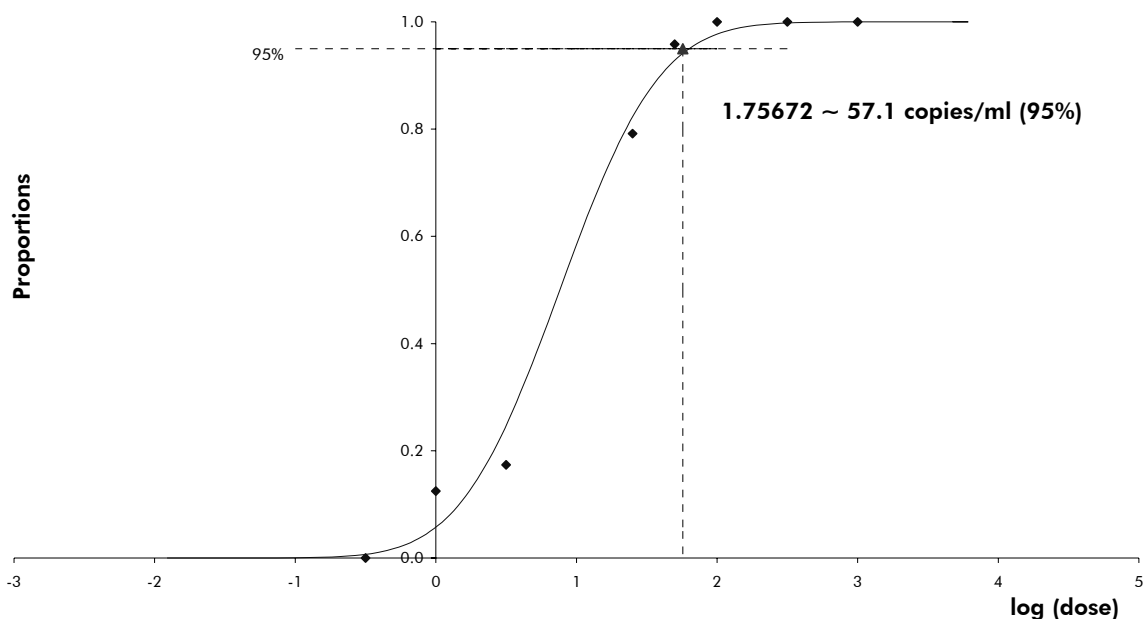


Figure 2. Probit analysis: CMV (Rotor-Gene 3000). Analytical sensitivity in consideration of the purification (QIAamp DSP Virus Kit, QIAGEN) of the *artus* CMV RG PCR Kit on the Rotor-Gene 3000.

The analytical sensitivity in consideration of the purification with EZ1[®] DSP Virus Kit (extraction volume: 0.4 ml, elution volume: 60 μ l) 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 1. None of the tested pathogens has been reactive. No cross-reactivities appeared with mixed infections.

Table 1. 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/ 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 2, page 12). In addition, precision data for quantitative results in copies/ μ l were determined using the corresponding C_T values (see Table 3, page 12). 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 $\geq 99\%$.

Table 2. 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 3. Precision data on basis of the quantitative results (in copies/ μ l)

	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

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 material safety data sheets (MSDSs), available from the product supplier.

- DNA isolation kit (see “DNA isolation”, page 15)
- 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 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.

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

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.

Please use only the recommended nucleic acid isolation kit (see “DNA isolation”, page 15) for sample preparation.

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

Specimen collection

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: Heparinized samples must not be used (see “Interfering substances”, page 15).

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 biological 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 biological material. The blood samples should be shipped cooled (2–8°C) and the separated plasma deep frozen (–15 to –30°C).

Interfering substances

Levels of ≤ 4.5 mg/dl bilirubin and ≤ 1000 mg/dl lipids 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 be used.

DNA isolation

The kits from QIAGEN shown in Table 4 are recommended 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 4. Purification kits validated for use with the *artus* CMV RG PCR Kit

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

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 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 μ l per 1 μ l elution volume. For example, using the QIAamp DSP Virus Kit, the DNA is eluted in 60 μ l Elution Buffer (AVE). Hence, 6 μ l 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 15) 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 19).

Quantitation

The enclosed quantitation standards (CMV 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).

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 19), and use this master mix for each quantitation standard (CMV QS 1–4).

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

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

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).

Protocol: PCR and Data Analysis

Important points before starting

- Before beginning the procedure, read “Important Notes”, pages 14–17.
- 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 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 16). In this case, prepare a master mix according to Table 5.

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

Table 5. 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 μ l	300 μ l
CMV Mg-Sol	5 μ l	60 μ l
CMV 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 CMV RG Master and CMV Mg-Sol. 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 exclusively to check for PCR inhibition)

Number of samples	1	12
CMV RG Master	25 μ l	300 μ l
CMV Mg-Sol	5 μ l	60 μ l
CMV 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 DNA (see Table 7). Correspondingly, 20 μ l of at least one of the quantitation standards (CMV QS 1–4) must be used as a positive control and 20 μ l of water (Water, PCR grade) as a negative control.**

Table 7. 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 CMV DNA, create a temperature profile according to the following steps.

Setting the general assay parameters	Figures 3, 4, 5
Initial activation of the hot-start enzyme	Figure 6
Amplification of the DNA (touchdown PCR)	Figure 7
Adjusting the fluorescence channel sensitivity	Figure 8
Starting the run	Figure 9

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 3). Check the “Locking Ring Attached” box and click “Next”.

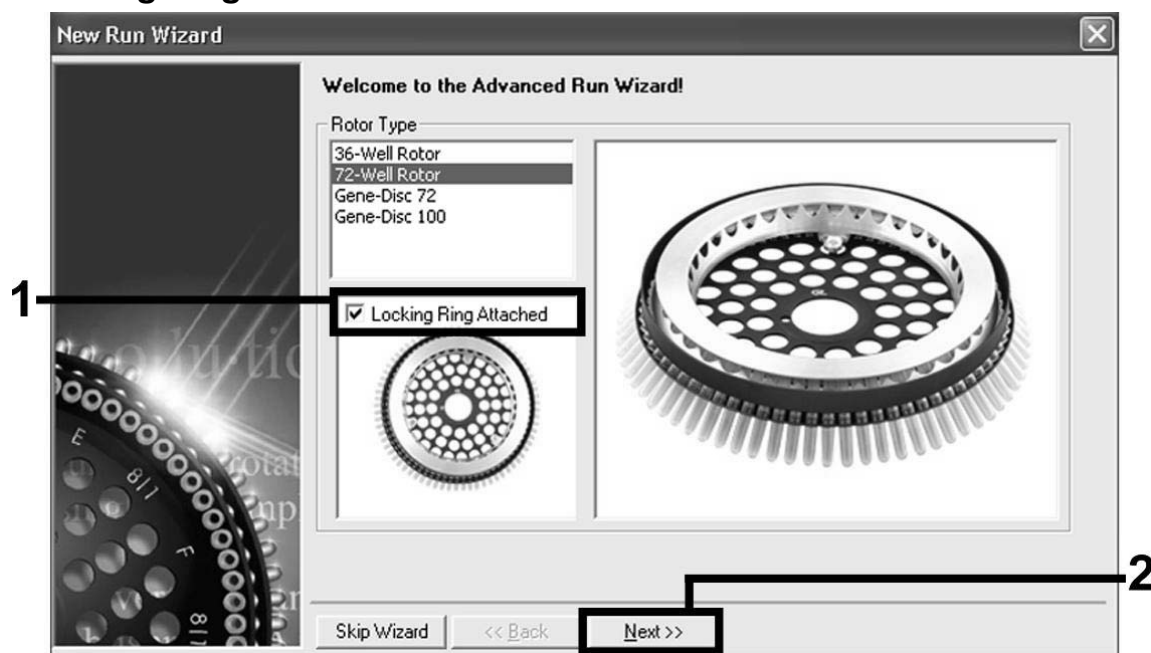


Figure 3. The “New Run Wizard” dialog box.

7. Select 50 for the PCR reaction volume and click “Next” (Figure 4).

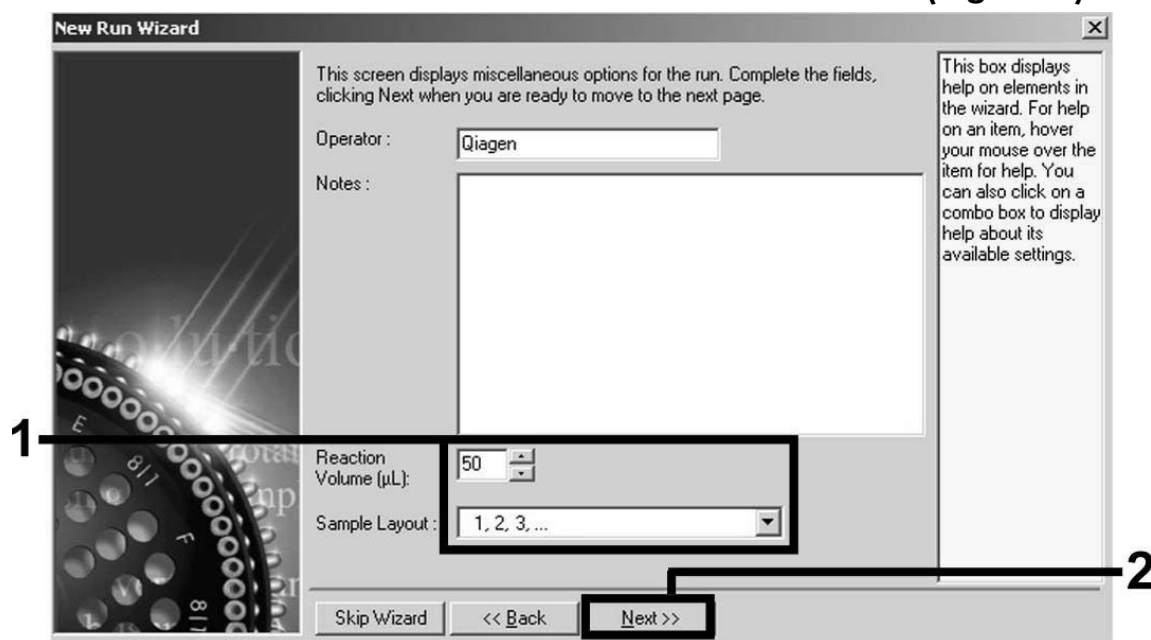


Figure 4. Setting the general assay parameters.

8. Click the “Edit Profile” button in the next “New Run Wizard” dialog box (Figure 5), and program the temperature profile as shown in Figures 5–7).

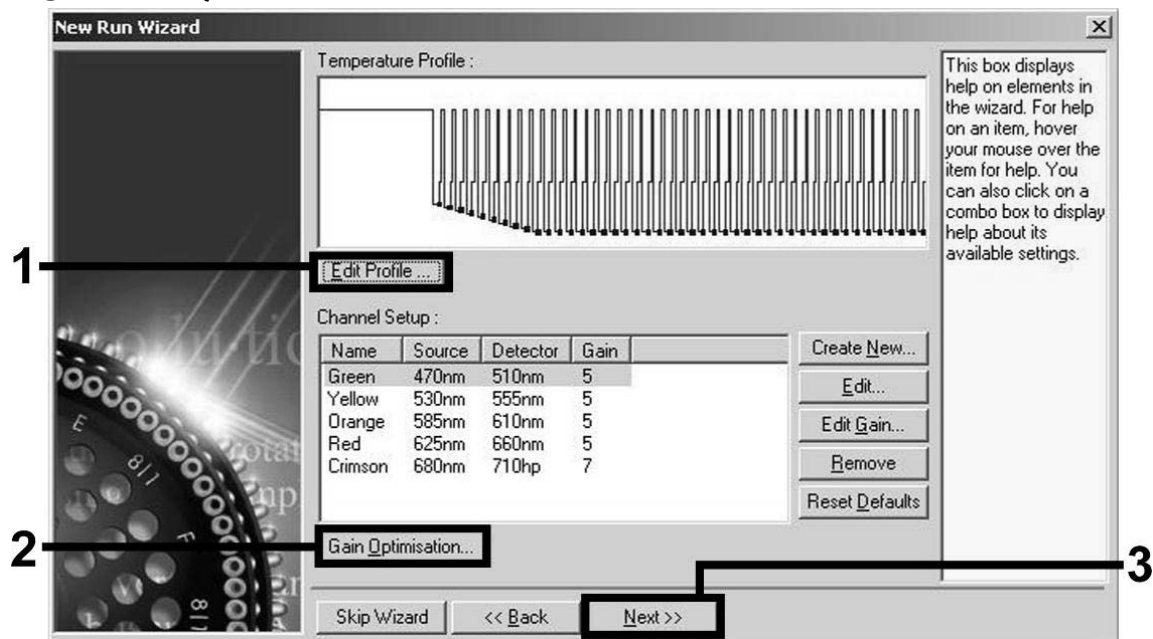


Figure 5. Editing the profile.

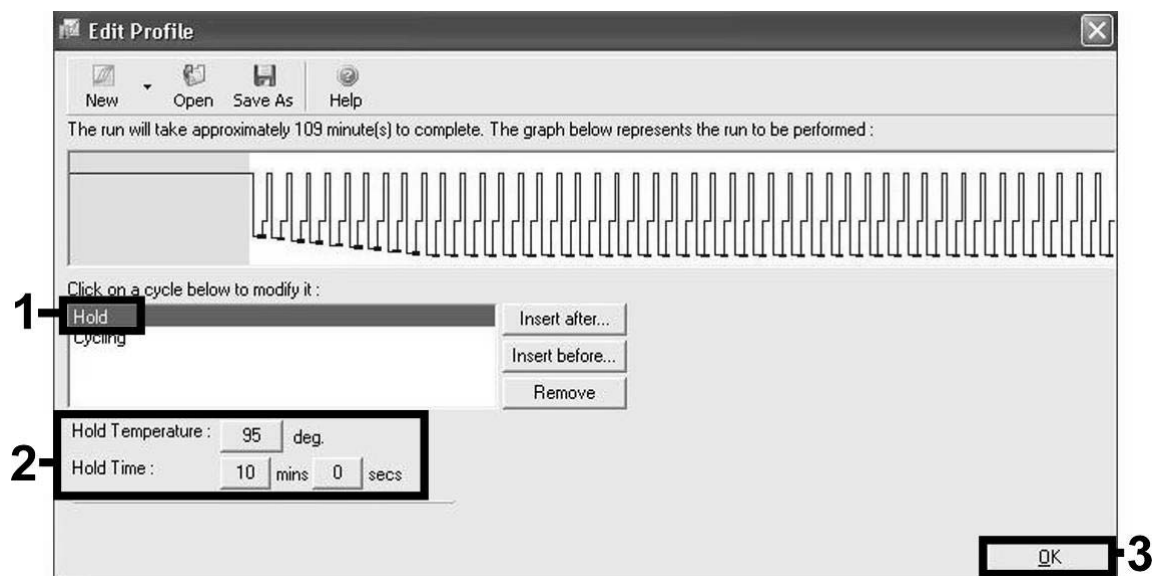


Figure 6. Initial activation of the hot-start enzyme.

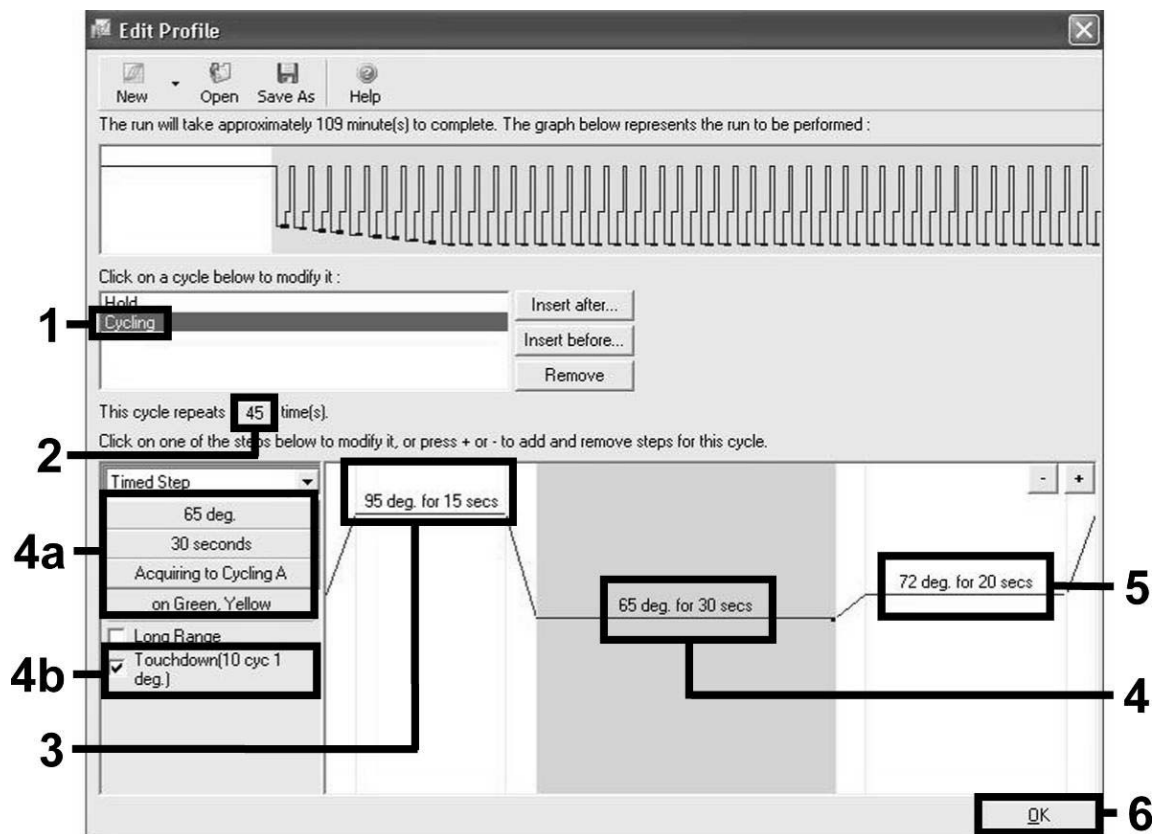


Figure 7. Amplification of the DNA. Make sure to activate the touchdown function for 10 cycles in the Annealing step. Note that, on the Rotor-Gene 3000, the software will define the fluorescence dyes as "FAM/Sybr, JOE".

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 5) 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 8).

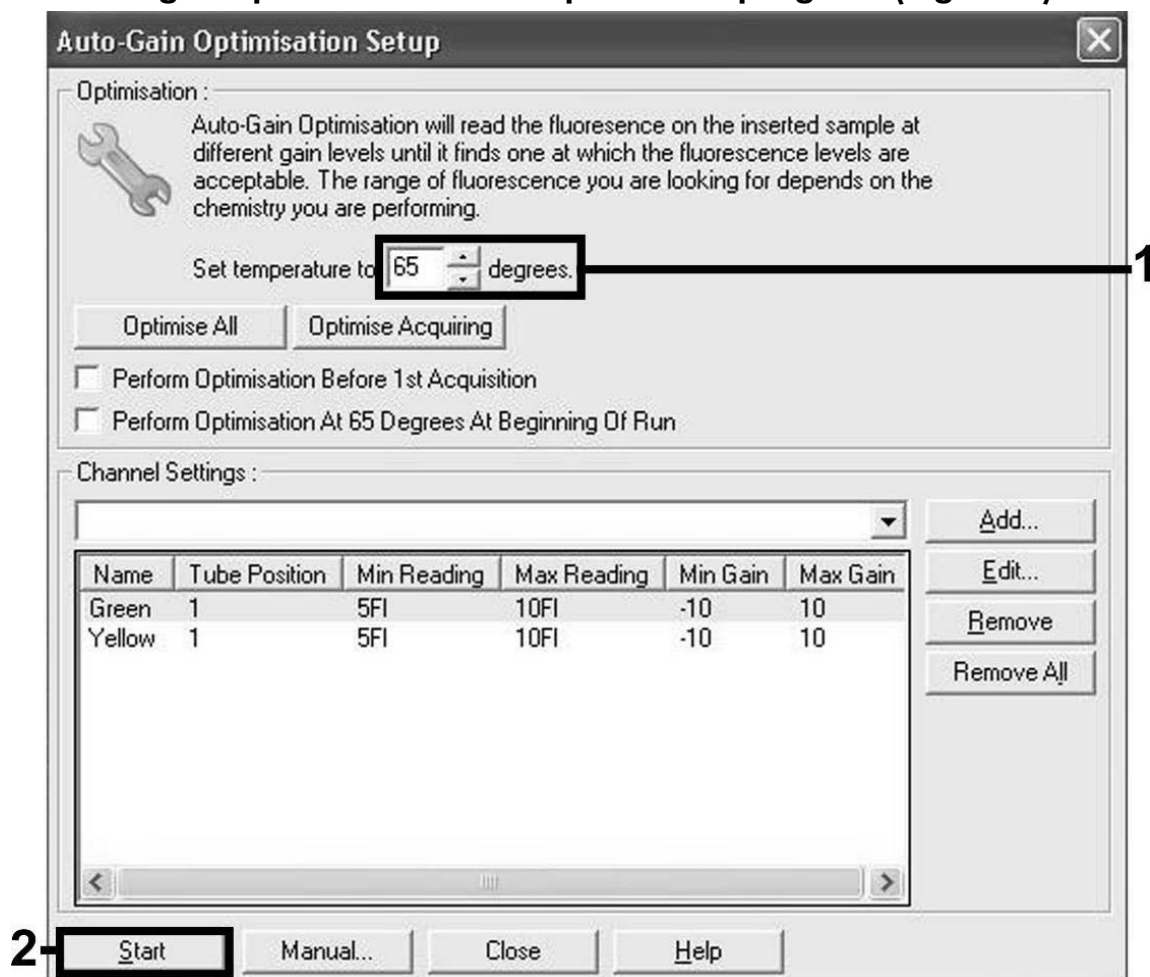


Figure 8. Adjusting the fluorescence channel sensitivity. Note that, on the Rotor-Gene 3000, the software will define the fluorescence dyes as "FAM/Sybr" and "JOE".

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 9). Click "Start Run".

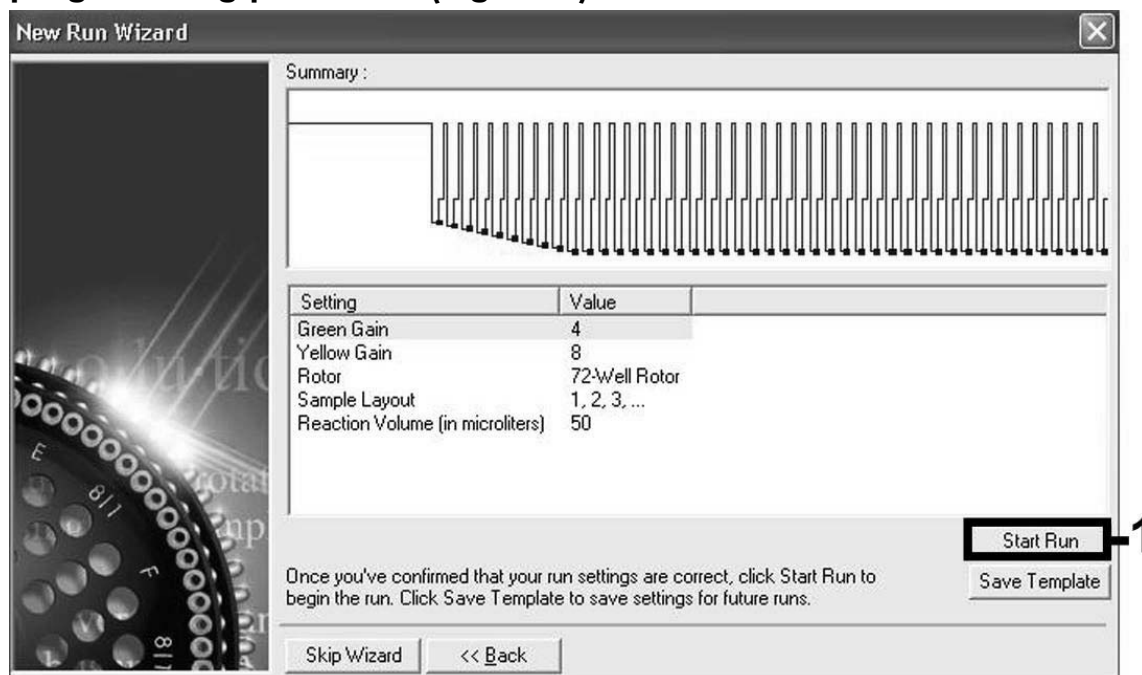


Figure 9. Starting the run. Note that, on the Rotor-Gene 3000, the software will define the fluorescence dyes as "FAM/Sybr" and "JOE".

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 10 and Figure 11.

11a. 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.

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 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.

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 28.

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

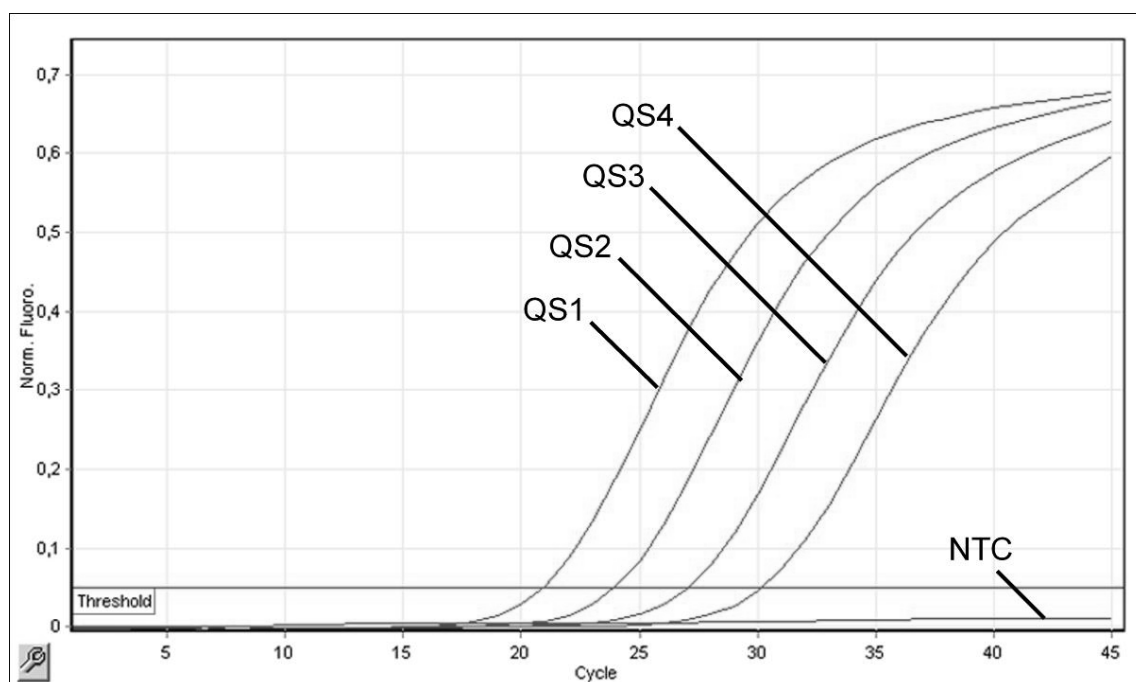


Figure 10. Detection of the quantitation standards (CMV QS 1–4) in fluorescence channel Cycling Green. NTC: No template control (negative control).

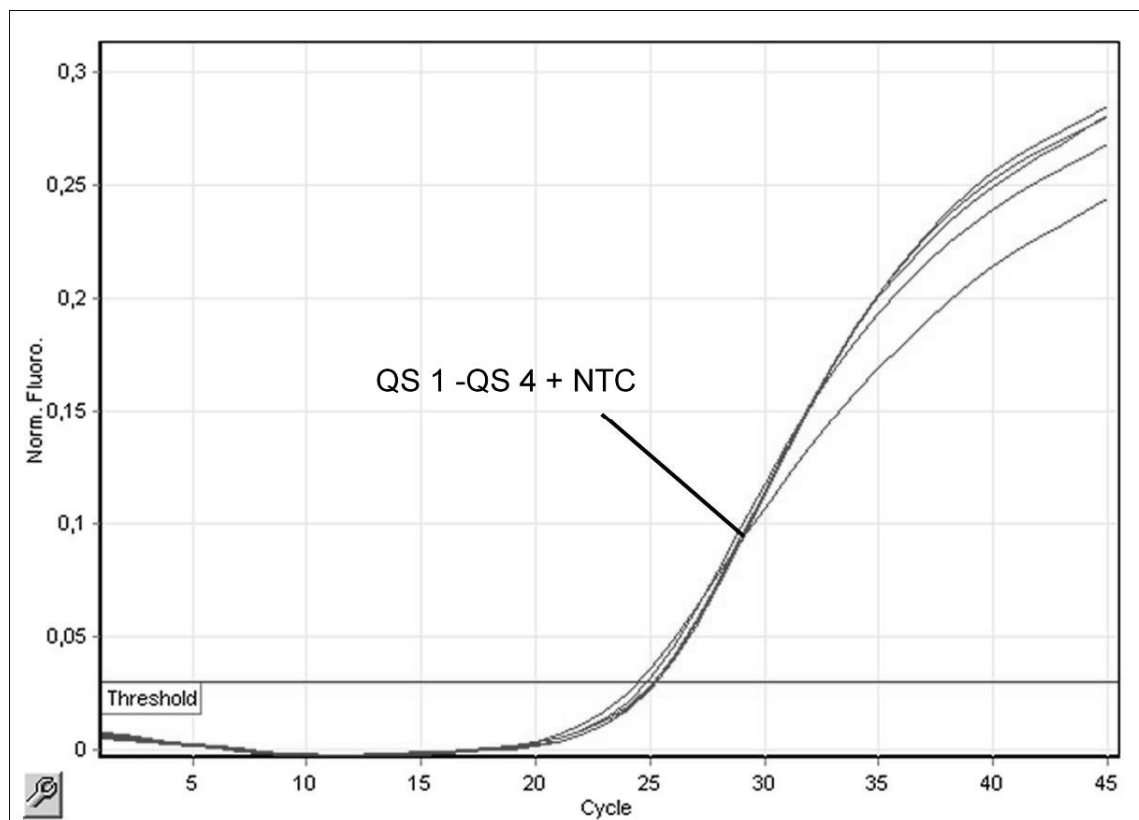


Figure 11. Detection of the internal control (IC) in fluorescence channel
Cycling Yellow with simultaneous amplification of the quantitation standards
(CMV QS 1–4). NTC: No template control (negative control).

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 18. |
| 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 18. |
| 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> 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

Weak or no signal of the internal control of a negative plasma sample subjected to purification using the QIAamp DSP Virus Kit ($C_T = 27 \pm 3$; 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 15) 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 <i>artus</i> 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. |

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 | <p>Repeat the PCR with new reagents in replicates.</p> <p>If possible, close the PCR tubes directly after addition of the sample to be tested.</p> <p>Make sure to pipet the positive controls last.</p> <p>Make sure that work space and instruments are decontaminated at regular intervals.</p> |
|---|--|

Comments and suggestions

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

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

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> CMV RG PCR Kit (24)	For 24 reactions: Master, Magnesium Solution, 4 Quantitation Standards, Internal Control, Water (PCR grade)	4503203
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
Rotor-Gene Q and accessories		
Rotor-Gene Q 5plex	Real-time PCR cyclers with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories, 1-year warranty on parts and labor	Inquire
Rotor-Gene Q 5plex HRM	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	Inquire
Rotor-Gene Q 6plex	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	Inquire
Rotor-Gene Q 2plex	Real-time PCR cycler with 2 channels (green, yellow), laptop computer, software, accessories, 1-year warranty on parts and labor	Inquire

Product	Contents	Cat. no.
Rotor-Gene Q 2plex HRM	Real-time PCR cyclers and High Resolution Melt analyzer with 2 channels (green, yellow) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor	Inquire
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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