

# artus<sup>®</sup> EBV RG PCR Kit Handbook



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

For use with Rotor-Gene<sup>®</sup> Q Instruments

**REF** 4501205

**HB** 1094041

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## Kit Contents





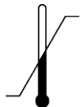


<b>artus EBV RG PCR Kit</b>			<b>(96)</b>
<b>Catalog no.</b>			<b>4501205</b>
<b>Number of reactions</b>			<b>96</b>
Blue	EBV RG Master		8 x 12 reactions
Red	EBV RG QS 1* (5 x 10 <sup>4</sup> copies/μl)	<b>QS</b>	200 μl
Red	EBV RG QS 2* (5 x 10 <sup>3</sup> copies/μl)	<b>QS</b>	200 μl
Red	EBV RG QS 3* (5 x 10 <sup>2</sup> copies/μl)	<b>QS</b>	200 μl
Red	EBV RG QS 4* (5 x 10 <sup>1</sup> copies/μl)	<b>QS</b>	200 μl
Green	EBV RG IC <sup>†</sup>	<b>IC</b>	2 x 1000 μl
White	Water (PCR grade)		1000 μl
	Handbook		1

\* Quantitation standard.

† Internal control.

## Symbols

 <N>	Contains sufficient for <N> tests
	Use by
	Catalog number
	Lot number
	Material number
	Components
	Contains

	Number
	Guanidine hydrochloride
	Isopropanol
	Global Trade Item Number
	Temperature limitation
	Manufacturer
	Consult instructions for use

## Storage

The components of the *artus* EBV RG PCR Kit should be stored at  $-30^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  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 safety data sheets (SDSs). These are available online in convenient and compact PDF format at [www.qiagen.com/safety](http://www.qiagen.com/safety) where you can find, view, and print the SDS for each QIAGEN<sup>®</sup> kit and kit component.

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

## Quality Control

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

## Introduction

The *artus* EBV RG PCR Kit constitutes a ready-to-use system for the detection of EBV DNA using polymerase chain reaction (PCR) on Rotor-Gene Q Instruments. The EBV RG Master contains reagents and enzymes for the specific amplification of a 97 bp region of the EBV 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* EBV 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 EBV PCR is not reduced. External positive controls (EBV RG QS 1–4) are supplied, which allow determination of the amount of viral DNA. For further information, see “Quantitation”, page 15.

## Principle

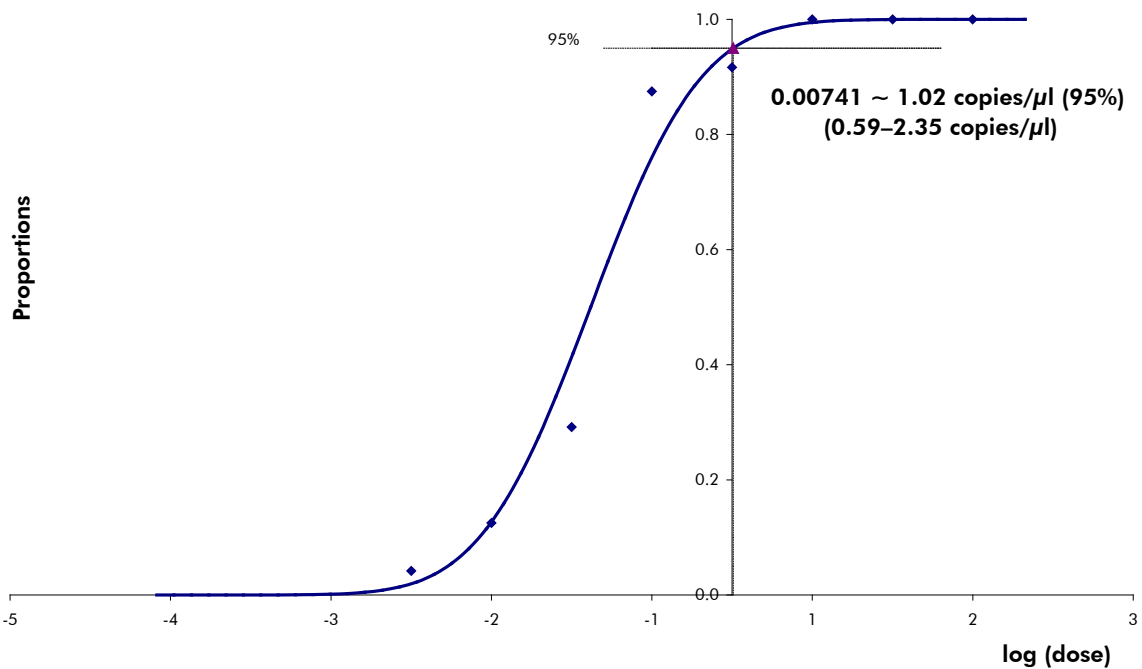
Pathogen detection by the polymerase chain reaction (PCR) is based on the amplification of specific regions of the pathogen genome. In real-time PCR the amplified product is detected via fluorescent dyes. These are usually linked to oligonucleotide probes that bind specifically to the amplified product.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

## Specifications

### Analytical sensitivity

To determine the analytical sensitivity of the *artus* EBV RG PCR Kit, a dilution series was set up from 31.6 to 0.01 and from 100 to nominal 0.03 EBV copy equivalents/ $\mu$ l and analyzed on the Rotor-Gene 6000 and Rotor-Gene 3000, respectively, in combination with the *artus* EBV 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* EBV RG PCR Kit in combination with the Rotor-Gene Q/6000 and the Rotor-Gene 3000 is 1.02 copies/ $\mu$ l ( $p = 0.05$ ) and 3.8 copies/ $\mu$ l ( $p = 0.05$ ), respectively. This means that there is a 95% probability that 1.02 copies/ $\mu$ l or 3.8 copies/ $\mu$ l will be detected.

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



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

## Specificity

The specificity of the *artus* EBV 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 published sequences in gene banks by sequence comparison analysis. The detectability of all relevant genotypes has thus been ensured.

Moreover, the specificity was checked with 6 different EBV negative serum samples. These did not generate any signals with the EBV specific primers and probes, which are included in the EBV RG Master.

A potential cross-reactivity of the *artus* EBV RG PCR Kit was tested using the control group listed in Table 1. None of the tested pathogens has been reactive.



**Table 1. Testing the specificity of the kit with potentially cross-reactive pathogens**

<b>Control group</b>	<b>EBV (Cycling Green or Cycling A.FAM)</b>	<b>Internal control (Cycling Yellow or Cycling A.JOE)</b>
Human herpesvirus 1 (herpes simplex virus 1)	–	+
Human herpesvirus 2 (herpes simplex virus 2)	–	+
Human herpesvirus 3 (varicella-zoster virus)	–	+
Human herpesvirus 5 (cytomegalovirus)	–	+
Human T cell leukemia virus 1	–	+
Human T cell leukemia virus 2	–	+

## Equipment and Reagents to Be Supplied by User

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

- DNA isolation kit (see “DNA isolation”, page 11)
- 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).

### DNA isolation

The kits from QIAGEN shown in Table 2 are recommended for viral DNA purification from the indicated human sample types for use with the *artus* EBV RG PCR Kit. Carry out the viral DNA purification according to the instructions in the kit handbooks.

**Table 2. Purification kits recommended for use with the *artus* EBV RG PCR Kit**

Sample material	Nucleic acid isolation kit	Catalog number (QIAGEN)	Carrier RNA
Serum, plasma, CSF	QIAamp® DNA Mini Kit (50)	51304	Not included
	QIAamp UltraSens® Virus Kit (50)	53704	Included
Blood cells	QIAamp DNA Blood Mini Kit (50)	51104	Not included
Plasma	EZ1® DSP Virus Kit (48)	62724	Included

**Note:** Blood collection tubes coated with anticoagulants may inhibit the PCR. However, these inhibitors will be eliminated by the use of the isolation kits listed above. We recommend to avoid the use of heparin blood.

**Note:** The *artus* EBV RG PCR Kit should not be used with phenol-based isolation methods.

### Using the QIAamp DNA Blood Mini Kit or QIAamp DNA Mini Kit

**Note:** The use of carrier RNA is critical for the extraction efficiency and, consequently, for DNA/RNA yield. Please note that the addition of carrier (RNA Homopolymer Poly[rA], not included in the QIAamp DNA Blood Mini Kit or the QIAamp DNA Mini Kit) is strongly recommended for the extraction of nucleic acids from cell-free body fluids and material with small amounts of DNA and RNA (e.g., CSF). In these cases, prepare carrier RNA as follows.

- Resuspend lyophilized carrier RNA (RNA Homopolymer Poly[rA], not included in the QIAamp DNA Blood Mini Kit or the QIAamp DNA Mini Kit) using the elution buffer (do not use lysis buffer) of the extraction kit (Buffer AE of the QIAamp DNA Mini Kit and QIAamp DNA Blood Mini Kit), and prepare a dilution with a concentration of 1 µg/µl. Divide this carrier RNA solution into a number of aliquots sufficient for your needs, and store them at –15 to –30°C. Avoid repeated thawing (>2 x) of a carrier RNA aliquot.
- Use 1 µg carrier RNA per 100 µl lysis buffer. For example, if the extraction protocol uses 200 µl lysis buffer, add 2 µl carrier RNA (1 µg/µl) directly into the lysis buffer (Buffer AL of the QIAamp DNA Mini Kit and QIAamp DNA Blood Mini Kit). Before the beginning of each extraction, a mixture of lysis buffer and carrier RNA (and internal control, where applicable, see “Internal control”, page 14) should be prepared freshly according to the pipetting scheme in Table 3.

**Table 3. Pipetting scheme for use with the QIAamp DNA Blood Mini Kit or the QIAamp DNA Mini Kit**

<b>Number of samples</b>	<b>1</b>	<b>12</b>
Buffer AL (lysis buffer)*	e.g., 200 µl	e.g., 2400 µl
Carrier RNA (1 µg/µl)	2 µl	24 µl
<b>Total volume</b>	<b>202 µl</b>	<b>2424 µl</b>
<b>Volume per extraction</b>	<b>200 µl</b>	<b>200 µl each</b>

\* Contains guanidine hydrochloride; see the kit handbook for safety information.

**Note:** Use the freshly prepared mixture of lysis buffer and carrier RNA immediately for extraction. Storage of the mixture is not possible.

**Note:** The internal control of the *artus* EBV RG PCR Kit can be used directly in the isolation procedure (see “Internal control”, page 14).

**Note:** We strongly recommend to perform the recommended centrifugation step 10 in the protocol (*QIAamp DNA Mini and Blood Mini Handbook*, Third Edition, April 2010, pages 29 and 32) to remove any residual ethanol. We recommend to increase the time of this centrifugation to 3 minutes.

We recommend to elute the DNA in 50  $\mu\text{l}$  elution buffer to get the highest sensitivity of the *artus* EBV RG PCR Kit.

### Using the QIAamp UltraSens Virus Kit

**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 UltraSens Virus Kit, we recommend the following procedure, which differs from the instructions in the kit handbook.

- Before the first use of the kit, resuspend the lyophilized carrier RNA in 310  $\mu\text{l}$  of the elution buffer (Buffer AVE) provided with the kit (final concentration 1  $\mu\text{g}/\mu\text{l}$ , do not use lysis buffer). Divide this carrier RNA solution into a number of aliquots sufficient for your needs, and store them at  $-15$  to  $-30^{\circ}\text{C}$ . Avoid repeated thawing ( $>2$  x) of a carrier RNA aliquot.
- Before the beginning of each extraction, a mixture of lysis buffer and carrier RNA (and internal control, where applicable, see “Internal control”, page 14) should be prepared freshly according to the pipetting scheme in Table 4.

**Table 4. Pipetting scheme for use with the QIAamp UltraSens Virus Kit**

<b>Number of samples</b>	<b>1</b>	<b>12</b>
Buffer AC (lysis buffer)*	800 $\mu\text{l}$	9600 $\mu\text{l}$
Carrier RNA (1 $\mu\text{g}/\mu\text{l}$ )	5.6 $\mu\text{l}$	67.2 $\mu\text{l}$
<b>Total volume</b>	<b>805.6 <math>\mu\text{l}</math></b>	<b>9667.2 <math>\mu\text{l}</math></b>
<b>Volume per extraction</b>	<b>800 <math>\mu\text{l}</math></b>	<b>800 <math>\mu\text{l}</math> each</b>

\* Contains isopropanol; see the kit handbook for safety information.

**Note:** Use the freshly prepared mixture of lysis buffer and carrier RNA immediately for extraction. Storage of the mixture is not possible.

**Note:** The internal control of the *artus* EBV RG PCR Kit can be used directly in the isolation procedure (see “Internal control”, page 14).

**Note:** We strongly recommend to perform the additional centrifugation described in step 14 of the protocol (*QIAamp UltraSens Virus Handbook*, April 2010, page 17) to remove any residual ethanol. We recommend to increase the time of this centrifugation to 3 minutes.

We recommend to elute the DNA in 50  $\mu$ l elution buffer to get the highest sensitivity of the *artus* EBV RG PCR Kit.

The QIAamp UltraSens Virus Kit allows a sample concentration. If you use sample material other than serum or plasma, add at least 50 % (v/v) of negative human plasma to the sample.

### Using the EZ1 DSP Virus Kit

**Note:** The use of carrier RNA is critical for extraction efficiency and, consequently, for DNA/RNA yield. Add the appropriate amount of carrier RNA to each extraction following the instructions in the *EZ1 DSP Virus Kit Handbook*.

**Note:** The internal control of the *artus* EBV RG PCR Kit can be used directly in the isolation procedure (see “Internal control”, below).

**Note:** We strongly recommend using the purified viral nucleic acids for PCR immediately after extraction using the EZ1 DSP Virus Kit. Alternatively, eluates can be stored for up to 3 days at 4°C before PCR analysis.

### Internal control

An internal control (EBV RG IC) is supplied. This allows the user both to monitor the DNA isolation procedure and to check for possible PCR inhibition. Using the EZ1 DSP Virus Kit for extraction, the internal control has to be added following the instructions in the *EZ1 DSP Virus Kit Handbook*. Using the QIAamp UltraSens Virus Kit, the QIAamp DNA Blood Mini Kit, or the QIAamp DNA Mini Kit, add the internal control to the isolation at a ratio of 0.1  $\mu$ l per 1  $\mu$ l elution volume. For example, using the QIAamp UltraSens Virus Kit, the DNA is eluted in 50  $\mu$ l Buffer AVE. Hence, 5  $\mu$ 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 11) 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.

The internal control can optionally be used exclusively to check for possible PCR inhibition. For this application, add the internal control directly to the EBV RG Master, as described in step 2b of the protocol (page 17).

## Quantitation

The enclosed quantitation standards (EBV RG QS 1–4) are treated as previously purified samples and the same volume is used (20  $\mu$ 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:** The quantitation standards are defined as copies/ $\mu$ 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 11–15.
- 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 (EBV 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 DNA isolation procedure and to check for possible PCR inhibition, follow step 2a. If you are using the internal control exclusively to check for PCR inhibition, follow step 2b.
  - 2a. The internal control has already been added to the isolation (see “Internal control”, page 14). 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)**

<b>Number of samples</b>	<b>1</b>	<b>12</b>
EBV RG Master	30 $\mu$ l	360 $\mu$ l
EBV RG IC	0 $\mu$ l	0 $\mu$ l
<b>Total volume</b>	<b>30 <math>\mu</math>l</b>	<b>360 <math>\mu</math>l</b>



**2b. The internal control must be added directly to the mixture of EBV RG Master. 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)**

<b>Number of samples</b>	<b>1</b>	<b>12</b>
EBV RG Master	30 $\mu$ l	360 $\mu$ l
EBV RG IC	2 $\mu$ l	24 $\mu$ l
<b>Total volume</b>	<b>32 <math>\mu</math>l*</b>	<b>384 <math>\mu</math>l*</b>

\* 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  $\mu$ l of the master mix into each PCR Tube. Then add 20  $\mu$ l of the eluted sample DNA (see Table 7). Correspondingly, 20  $\mu$ l of at least one of the quantitation standards (EBV RG QS 1–4) must be used as a positive control and 20  $\mu$ l of water (Water, PCR grade) as a negative control.**

**Table 7. Preparation of PCR assay**

<b>Number of samples</b>	<b>1</b>	<b>12</b>
Master mix	30 $\mu$ l	30 $\mu$ l each
Sample	20 $\mu$ l	20 $\mu$ l each
<b>Total volume</b>	<b>50 <math>\mu</math>l</b>	<b>50 <math>\mu</math>l each</b>

**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.**

- For the detection of EBV DNA, create a temperature profile according to the following steps.

Setting the general assay parameters	Figures 2, 3, 4
Initial activation of the hot-start enzyme	Figure 5
Amplification of the DNA (touchdown PCR)	Figure 6
Adjusting the fluorescence channel sensitivity	Figure 7
Starting the run	Figure 8

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.

- First, open the “New Run Wizard” dialog box (Figure 2). Check the “Locking Ring Attached” box and click “Next”.

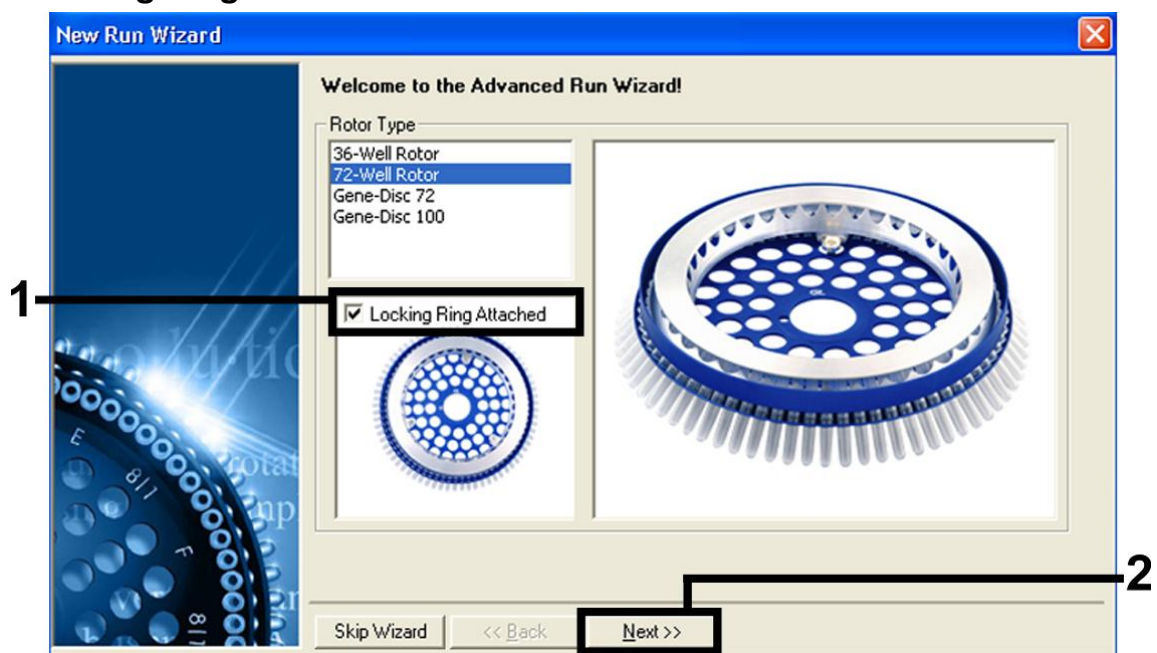


Figure 2. The “New Run Wizard” dialog box.

7. Select 50 for the PCR reaction volume and click "Next" (Figure 3).

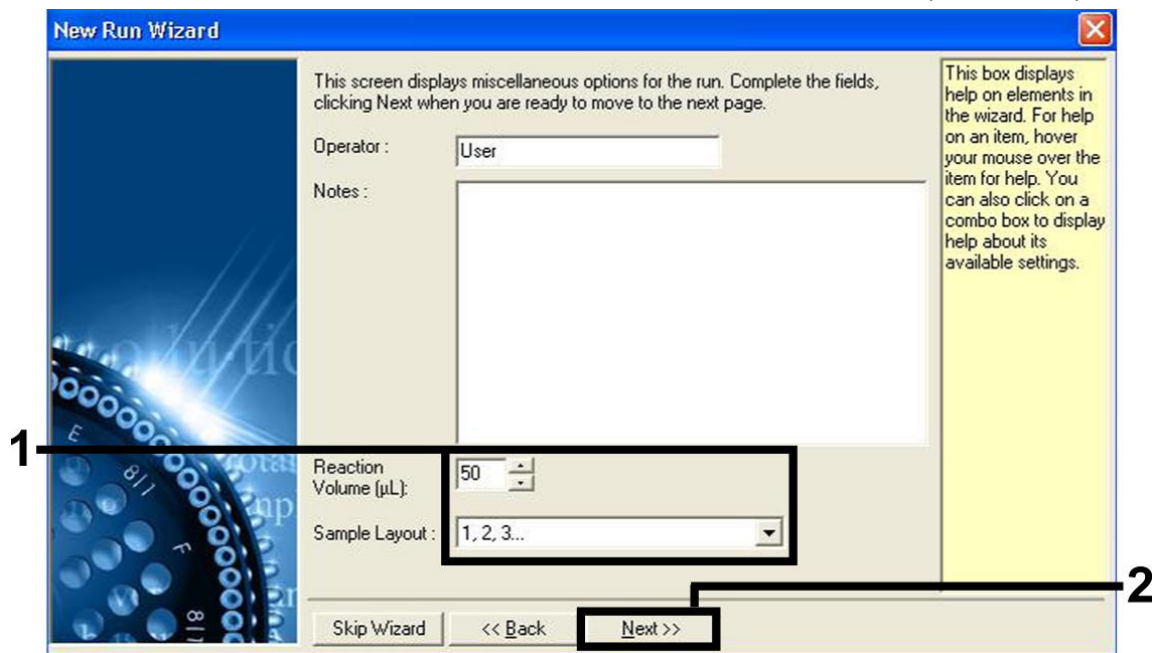


Figure 3. Setting the general assay parameters.

8. Click the "Edit Profile" button in the next "New Run Wizard" dialog box (Figure 4), and program the temperature profile as shown in Figures 4–6).

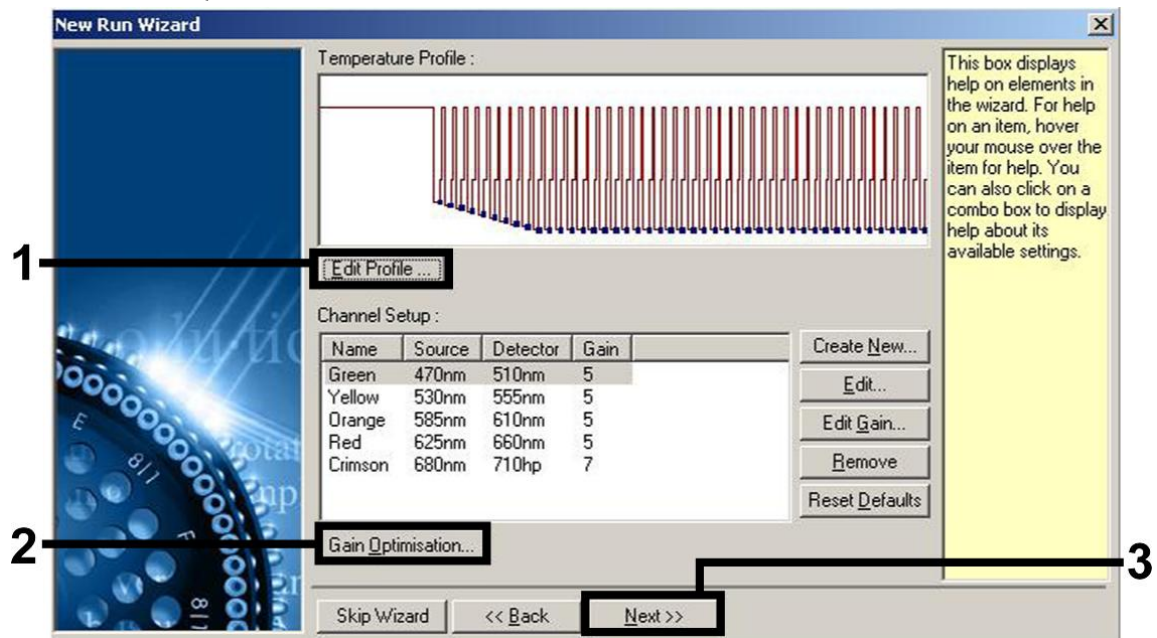
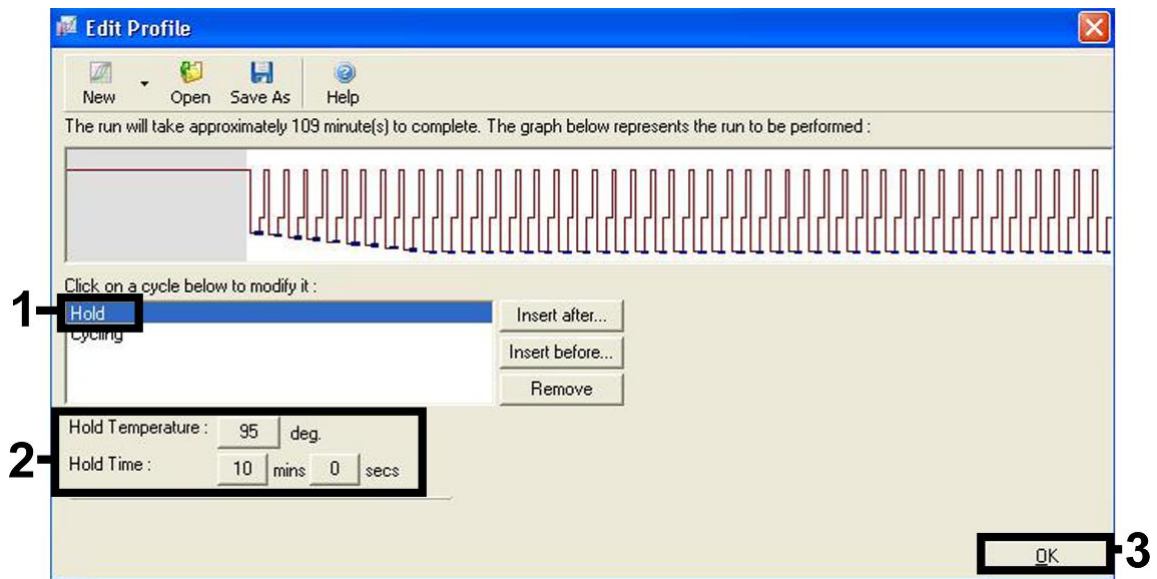
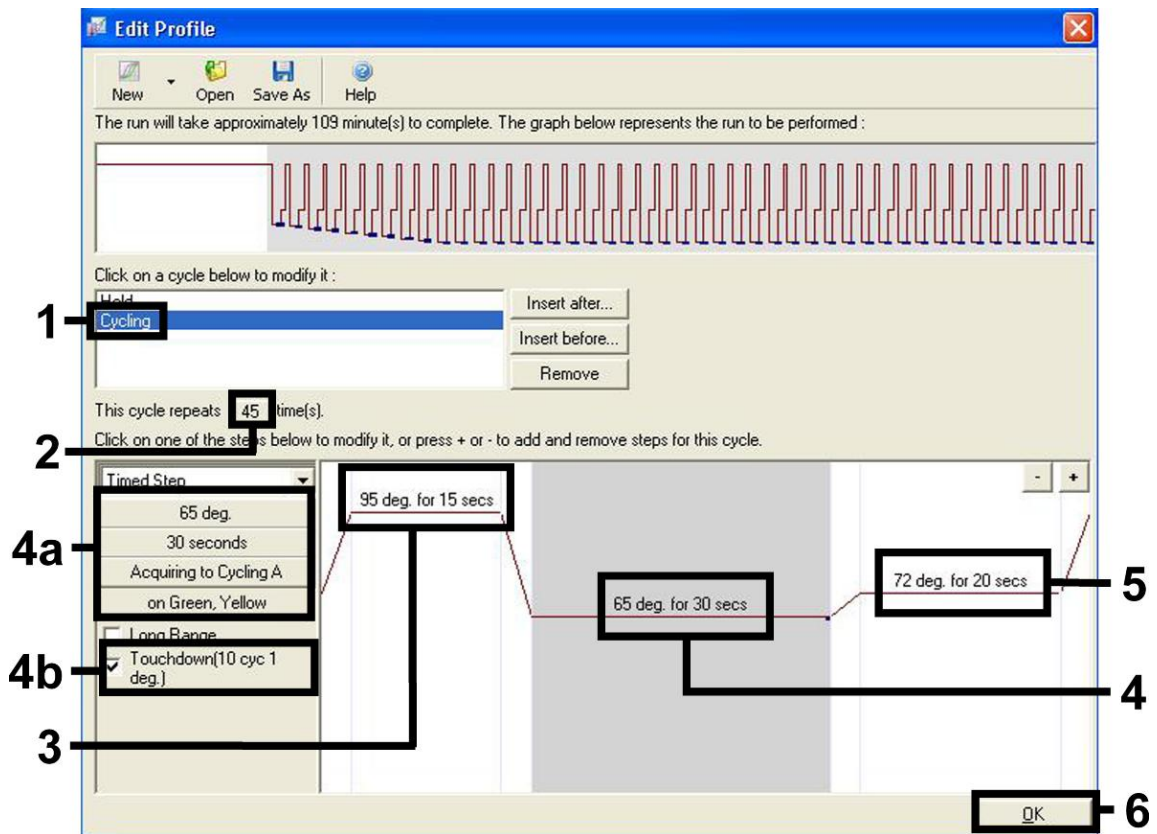


Figure 4. Editing the profile.

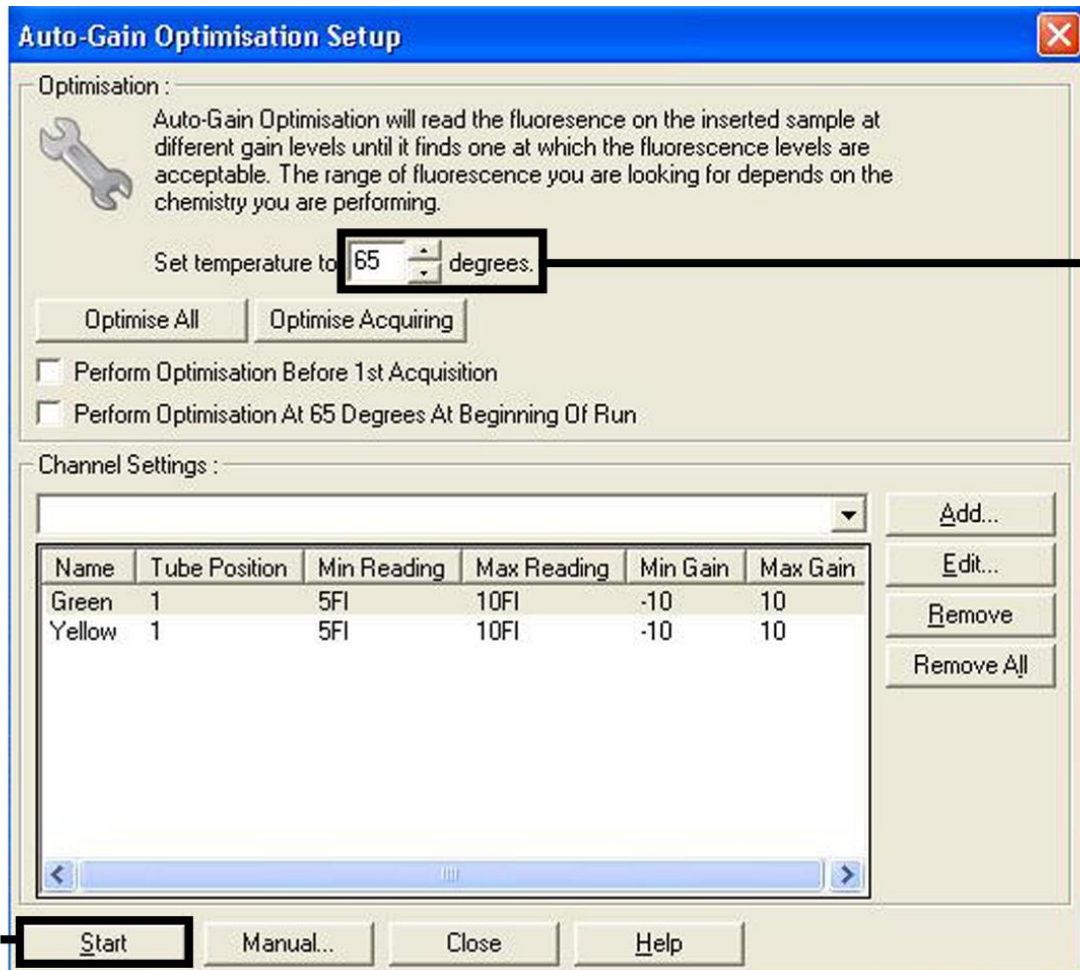


**Figure 5. Initial activation of the hot-start enzyme.**



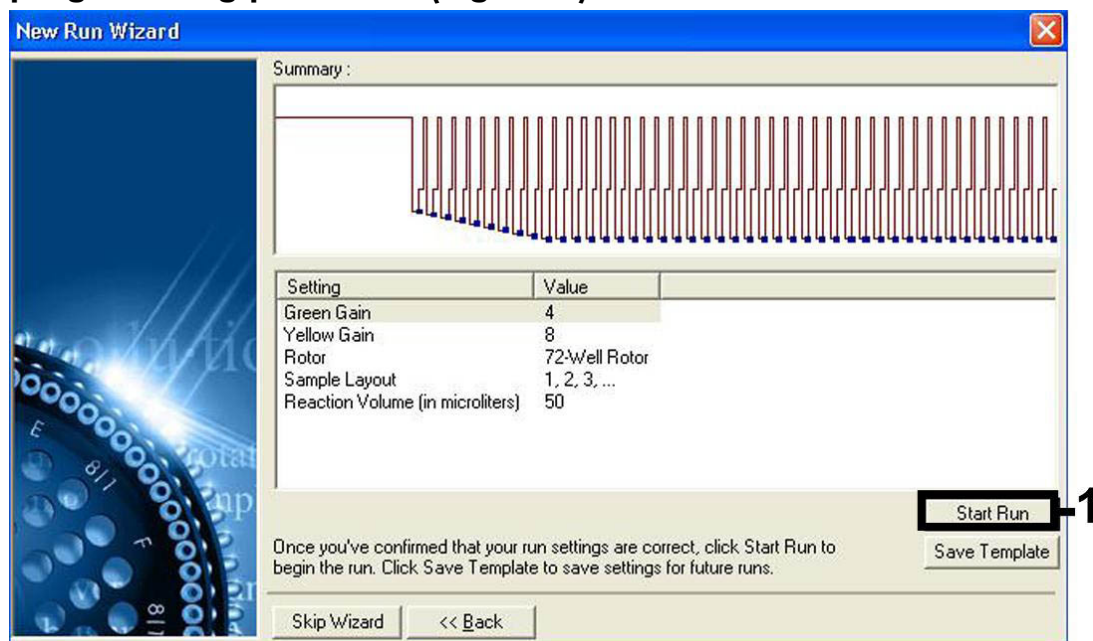
**Figure 6. Amplification of the DNA.** Make sure to activate the touchdown function for 10 cycles in the Annealing step. Note that, on the Rotor-Gene 3000, the software will define the fluorescence dyes as "FAM/Sybr, JOE".

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 4) 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 7).



**Figure 7. 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 8). Click "Start Run".



**Figure 8. 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 9 and Figure 10.

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

In this case, the detection of a signal in the Cycling Yellow channel is dispensable, since high initial concentrations of EBV 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 EBV DNA is detectable. It can be considered negative.**

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

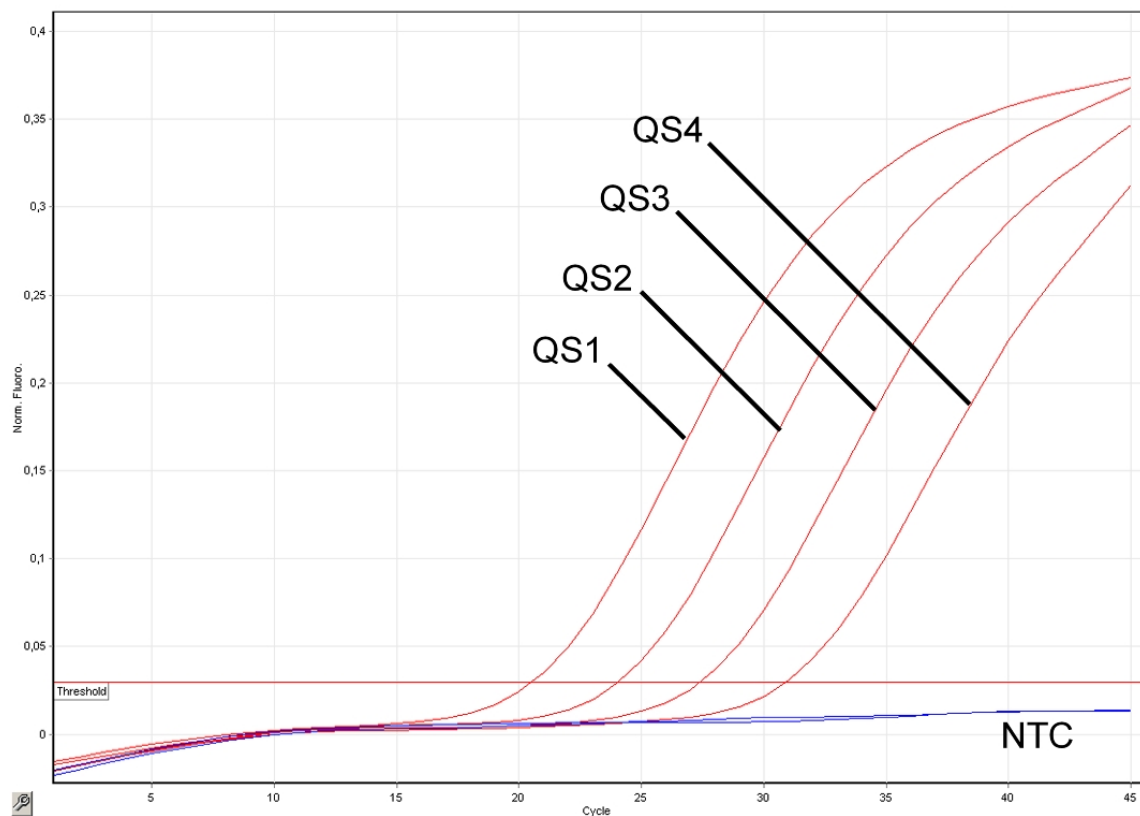
**Note:** On the Rotor-Gene 3000, the relevant channels are Cycling A.JOE for the internal control and lack of a signal for Cycling A.FAM.

**11c. No signal is detected in the Cycling Green or in the Cycling Yellow channels.**

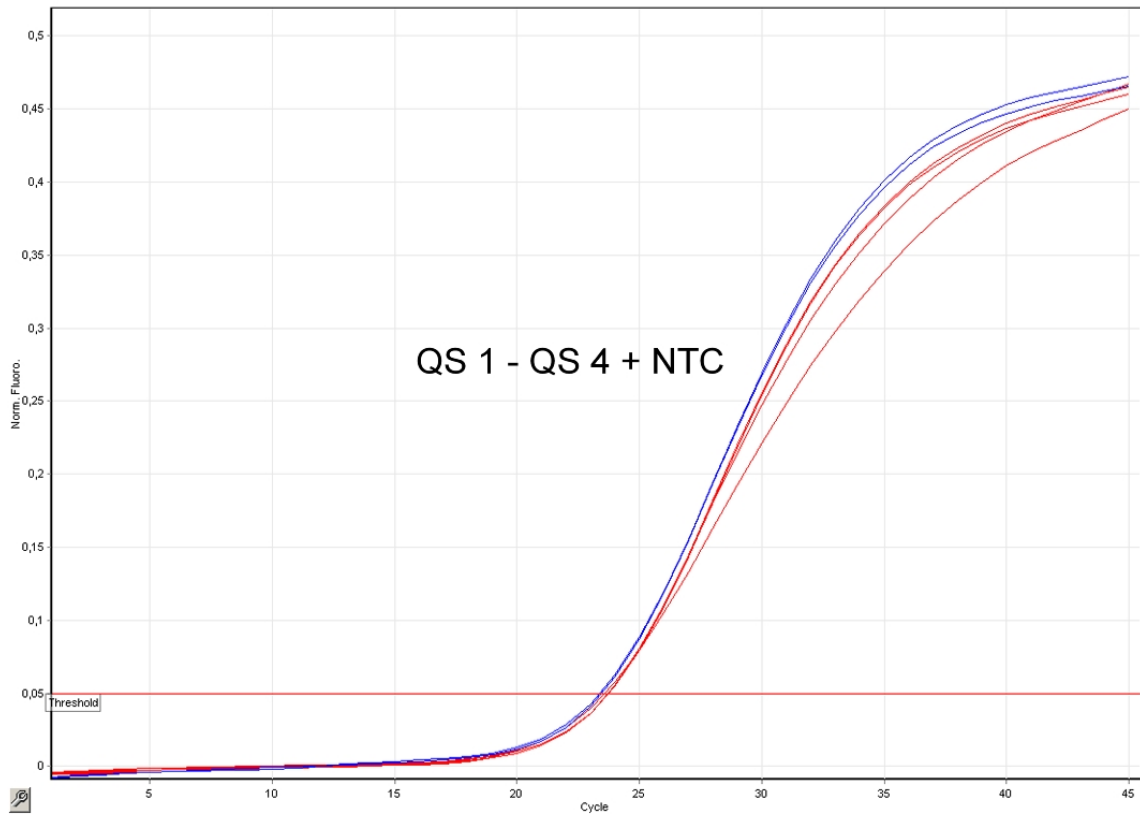
**No result can be concluded.**

Information regarding error sources and their solution can be found in “Troubleshooting Guide”, page 25.

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



**Figure 9. Detection of the quantitation standards (EBV RG QS 1–4) in fluorescence channel Cycling Green. NTC: No template control (negative control).**



**Figure 10. Detection of the internal control (IC) in fluorescence channel Cycling Yellow with simultaneous amplification of the quantitation standards (EBV RG 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](http://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](http://www.qiagen.com)).

### Comments and suggestions

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#### **No signal with positive controls (EBV 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 EBV 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 16.   |
| 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 16.  |
| 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> EBV 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 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. |
|---|--|

## Comments and suggestions

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- b) The PCR was inhibited      Make sure that you use the recommended isolation method and closely follow the manufacturer's instructions.
- When using the QIAamp DNA Mini Kit, QIAamp DNA Blood Mini Kit, or QIAamp UltraSens Virus Kit, make sure that during the DNA isolation the recommended additional centrifugation step has been carried out before the elution in order to remove any residual ethanol (see "DNA isolation", pages 12 and 13).
- 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 11) 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* EBV 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      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.

## Comments and suggestions

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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](http://www.qiagen.com/RefDB/search.asp) or contact QIAGEN Technical Services or your local distributor.

## Ordering Information

Product	Contents	Cat. no.
<i>artus</i> EBV RG PCR Kit (96)	For 96 reactions: Master, 4 Quantitation Standards, Internal Control, Water (PCR grade)	4501205
<b>EZ1 DSP Virus Kit — for automated, simultaneous purification of viral DNA and RNA from 1–14 serum, plasma, or CSF samples</b>		
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
<b>QIAamp DNA Mini Kit — for purification of genomic and viral DNA from tissues and other samples</b>		
QIAamp DNA Mini Kit (50)	For 50 DNA preps: 50 QIAamp Mini Spin Columns, QIAGEN Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51304
<b>QIAamp UltraSens Virus Kit — for concentration and isolation of viral DNA and RNA from serum and plasma</b>		
QIAamp UltraSens Virus Kit (50)	For 50 viral nucleic acid preps: 50 QIAamp Mini Spin Columns, Proteinase K, Carrier RNA, Collection Tubes (2 ml), Buffers	53704
<b>QIAamp DNA Blood Mini Kit — for purification of up to 12 µg genomic, mitochondrial, or viral DNA from blood and related body fluids</b>		
QIAamp DNA Blood Mini Kit (50)	For 50 DNA minipreps: 50 QIAamp Mini Spin Columns, QIAGEN Protease, Reagents, Buffers, Collection Tubes (2 ml)	51104
<b>Rotor-Gene Q and accessories</b>		
Rotor-Gene Q 5plex Platform	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training not included	9001570

<b>Product</b>	<b>Contents</b>	<b>Cat. no.</b>
Rotor-Gene Q 5plex System	Real-time PCR cyclers with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9001640
Rotor-Gene Q 5plex HRM Platform	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training not included	9001580
Rotor-Gene Q 5plex HRM System	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9001650
Rotor-Gene Q 6plex Platform	Real-time PCR instrument with 6 channels (blue, green, yellow, orange, red, crimson), including laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training not included	9001590
Rotor-Gene Q 6plex System	Real-time PCR instrument with 6 channels (blue, green, yellow, orange, red, crimson), including laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9001660
Rotor-Gene Q 2plex Platform	Real-time PCR cycler and High Resolution Melt analyzer with 2 channels (green, yellow) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training not included	9001550

<b>Product</b>	<b>Contents</b>	<b>Cat. no.</b>
Rotor-Gene Q 2plex System	Real-time PCR cycler with 2 channels (green, yellow), laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9001620
Rotor-Gene Q 2plex HRM Platform	Real-time PCR cycler and High Resolution Melt analyzer with 2 channels (green, yellow) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training not included	9001560
Rotor-Gene Q 2plex HRM System	Real-time PCR cycler and High Resolution Melt analyzer with 2 channels (green, yellow) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9001630
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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**Notes**



**Notes**

**Notes**

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