artus® BK Virus RG PCR Kit Handbook



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

For use with Rotor-Gene® Q Instruments



HB 1057575

QIAGEN GmbH, QIAGEN Strasse 1, D-40724 Hilden

MAT 1057575



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Contents

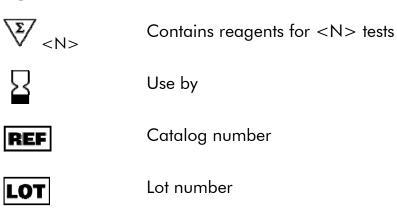
Kit Contents	4
Symbols	4
Storage	5
Product Use Limitations	5
Quality Control	5
Product Warranty and Satisfaction Guarantee	5
Technical Assistance	6
Safety Information	7
Introduction	8
Principle	8
Pathogen information	8
Equipment and Reagents to Be Supplied by User	9
Important Notes	10
General precautions	10
DNA isolation	10
Internal control	10
Quantitation	11
Protocol	
■ PCR and Data Analysis	12
Troubleshooting Guide	21
References	23
Ordering Information	24

Kit Contents

artus BK Virus RG PCR Kit Catalog no. Number of reactions		(96) 4514205 96	
Yellow	BK Virus RG Mg-Sol*	Mg-Sol	$400~\mu$ l
Red	BK Virus RG QS 1 [†] (1 x 10 ⁴ copies/μl)	QS	200 μl
Red	BK Virus RG QS2 [†] (1 x 10 ³ copies/µl)	QS	200 μl
Red	BK Virus RG QS3 [†] (1 x 10 ² copies/µl)	QS	200 μl
Red	BK Virus RG QS4 [†] (1 x 10 ¹ copies/µl)	QS	200 μl
Green	BK Virus RG IC [‡]	IC	2 x 1000 μl
White	Water (PCR grade)		1000 μ l
	Handbook	HB	1

^{*} Magnesium solution.

Symbols



Material number

[†] Quantitation standard.

[‡] Internal control.

COMP

Components

Contains

Number

Temperature limitations

Legal manufacturer

Refer to information given in the handbook

Storage

The components of the artus BK Virus 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 $2-8^{\circ}$ 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.

Quality Control

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

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

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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.

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Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Introduction

The artus BK Virus RG PCR Kit constitutes a ready-to-use system for the detection of BK virus DNA using polymerase chain reaction (PCR) on Rotor-Gene Q Instruments. The BK Virus RG Master contains reagents and enzymes for the specific amplification of a 274 bp region of the BK virus genome, and for the direct detection of the specific amplicon in fluorescence channel Cycling Green of the Rotor-Gene Q or Rotor-Gene 6000.

In addition, the *artus* BK Virus 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 Orange of the Rotor-Gene Q or Rotor-Gene 6000. The detection limit of the analytical BK Virus PCR is not reduced. External positive controls (BK Virus RG QS 1–4) are supplied, which allow the determination of the amount of viral DNA. For further information, see "Quantitation", page 11.

Principle

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

Pathogen information

BK virus (BKV) is a DNA virus belonging to the polyomaviruses. Primary infection occurs mainly during childhood and is usually asymptomatic. The seroprevalence in adults is up to 90%.

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

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 10)
- 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 Orange
- Rotor-Gene Q software version 1.7.94 or higher (Rotor-Gene 6000 software version 1.7.65)
- Strip Tubes and Caps, 0.1 ml, for use with 72-well rotor (cat. no. 981103 or 981106)
- Alternatively: PCR Tubes, 0.2 ml, for use with 36-well rotor (cat. no. 981005 or 981008)
- Cooling block (Loading Block 72 x 0.1 ml Tubes, cat. no. 9018901, or Loading Block 96 x 0.2 ml Tubes, cat. no. 9018905)

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

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

Important Notes

General precautions

The user should always pay attention to the following:

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

DNA isolation

The EZ1 DSP Virus Kit (QIAGEN, cat. no. 62724) may be used for viral nucleic acid purification from human plasma or urine, using the EZ1 Advanced DSP Virus Card (cat. no. 9018306) on the EZ1 Advanced (cat. no. 9001411), for use with the artus BK Virus RG PCR Kit. Carry out the viral DNA purification according to the instructions in the EZ1 DSP Virus Kit Handbook.

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

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* BK Virus RG PCR Kit can be used directly in the isolation procedure (see "Internal control", page 10).

Note: We strongly recommend to use 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 (BK Virus 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 μ l per 1 μ l elution volume. For example, using the EZ1 DSP Virus Kit, if the viral

nucleic acids are eluted in 60 μ l Elution Buffer (AVE), then 6 μ l of the internal control should be added initially.

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.

The internal control can optionally be used exclusively to check for possible PCR inhibition. For this application, add the internal control directly to the mixture of BK Virus RG Master and BK Virus RG Mg-Sol, as described in step 2b of the protocol (page 13).

Quantitation

The enclosed quantitation standards (BK Virus RG QS 1–4) are treated as previously purified samples and the same volume is used (15 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: 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:

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

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).

Note: A guideline for the quantitative analysis of pathogen detection systems on Rotor-Gene Instruments is available from QIAGEN Technical Services ("Technical Note for quantitation on Rotor-Gene Instruments").

Protocol: PCR and Data Analysis

Important points before starting

- Before beginning the procedure, read "Important Notes", pages 10–11.
- 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 (BK Virus RG QS 1-4) for each PCR run.

Things to do before starting

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

Procedure

- 1. Place the desired number of PCR tubes into the adapters of the cooling block.
- 2. If you are using the internal control to monitor the 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 10). In this case, prepare a master mix according to Table 1.

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

Table 1. Preparation of master mix (internal control used to monitor DNA isolation and check for PCR inhibition)

Number of samples	1	12
BK Virus RG Master	7 μl	84 μl
BK Virus RG Mg-Sol	$3~\mu$ l	36 <i>μ</i> Ι
BK Virus RG IC	0 μ l	0 μΙ
Total volume	10 <i>μ</i> l	120 μΙ

2b. The internal control must be added directly to the mixture of BK Virus RG Master and BK Virus RG Mg-Sol. 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 exclusively to check for PCR inhibition)

Number of samples	1	12
BK Virus RG Master	7 μl	84 μl
BK Virus RG Mg-Sol	3 <i>µ</i> l	36 <i>μ</i> l
BK Virus RG IC	1.5 <i>μ</i> l	18 <i>μ</i> l
Total volume	11.5 <i>µ</i> l*	138 <i>μ</i> l*

^{*} 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 10 μ l of the master mix into each PCR tube. Then add 15 μ l of the eluted sample DNA (see Table 3). Correspondingly, 15 μ l of at least one of the quantitation standards (BK Virus RG QS 1–4) must be used as a positive control and 15 μ l of water (Water, PCR grade) as a negative control.

Table 3. Preparation of PCR assay

Number of samples	1	12
Master mix	10 <i>μ</i> l	10 <i>μ</i> l each
Sample	15 <i>μ</i> l	15 <i>μ</i> l each
Total volume	25 μl	25 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 BK virus 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	Figure 5
Adjusting the fluorescence channel sensitivity	Figure 6
Starting the run	Figure 7

All specifications refer to the Rotor-Gene Q software version 1.7.94 and Rotor-Gene 6000 software version 1.7.65. 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.

6. First, open the "New Run Wizard" dialog box (Figure 1). Check the "Locking Ring Attached" box and click "Next".

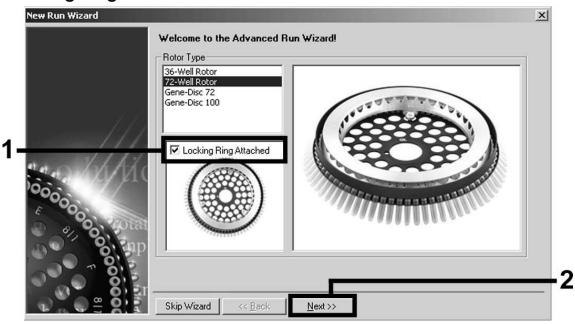


Figure 1. The "New Run Wizard" dialog box.

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

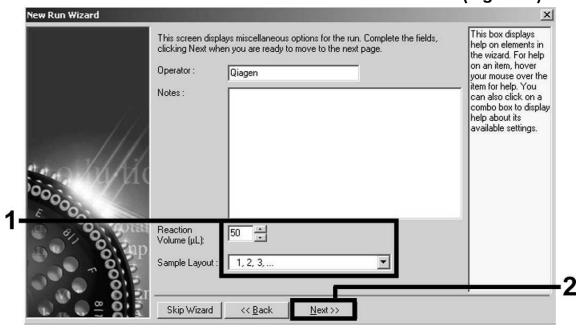


Figure 2. Setting the general assay parameters.

Note: Although the physical reaction volume is $25 \mu l$, make sure to select 50 for the reaction volume in the Rotor-Gene software.

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).

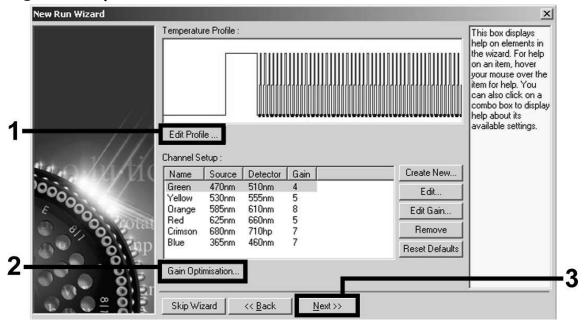


Figure 3. Editing the profile.

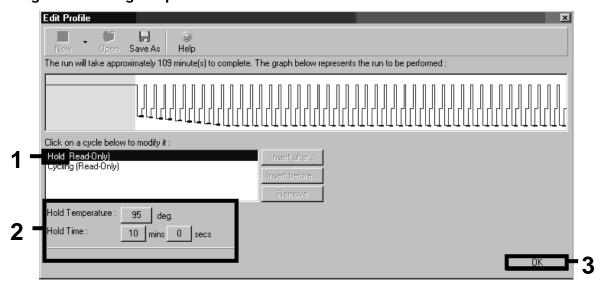


Figure 4. Initial activation of the hot-start enzyme.

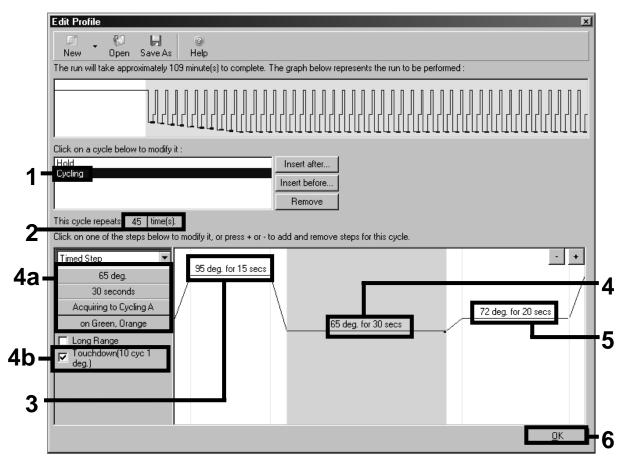


Figure 5. Amplification of the DNA. Make sure to activate the touchdown function for 10 cycles in the Annealing step.

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).

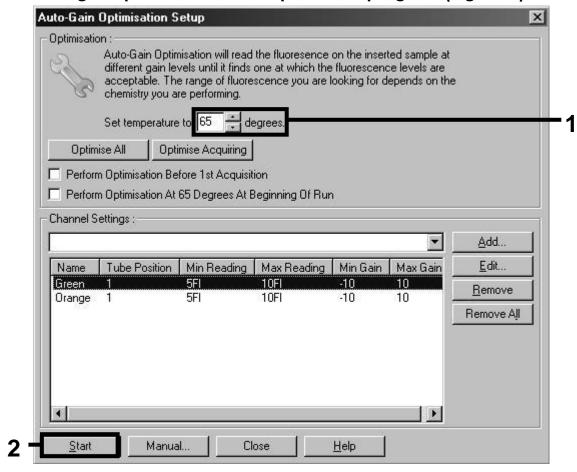


Figure 6. Adjusting the fluorescence channel sensitivity.

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".

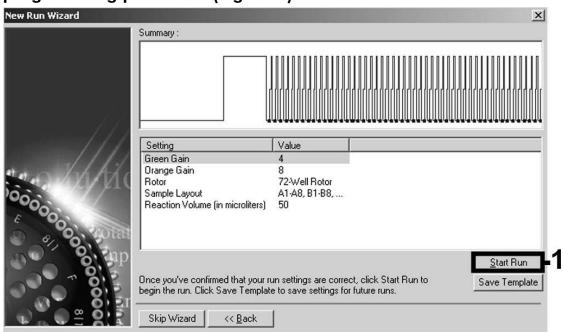


Figure 7. Starting the run.

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 8 and Figure 9.

11a.A signal is detected in fluorescence channel Cycling Green.

The result of the analysis is positive: the sample contains BK virus DNA.

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

11b.In fluorescence channel Cycling Green no signal is detected. At the same time, a signal from the internal control appears in the Cycling Orange channel.

In the sample no BK virus DNA is detectable. It can be considered negative.

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

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

No result can be concluded.

Information regarding error sources and their solution can be found in "Troubleshooting Guide", page 21.

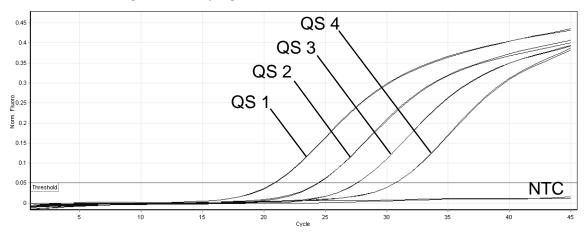


Figure 8. Detection of the quantitation standards (BK Virus RG QS 1–4) in fluorescence channel Cycling Green. NTC: No template control (negative control).

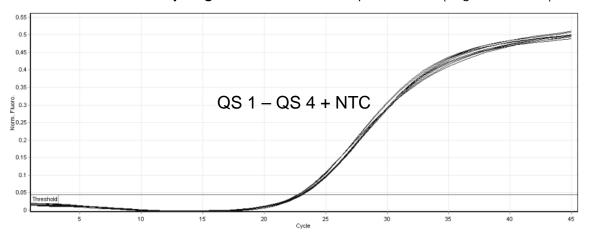


Figure 9. Detection of the internal control (IC) in fluorescence channel Cycling Orange with simultaneous amplification of the quantitation standards (BK Virus RG QS 1–4). NTC: No template control (negative control).

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. 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 (BK Virus RG QS 1-4) in fluorescence channel Cycling Green

 a) The selected fluorescence channel for PCR data analysis does not comply with the protocol For data analysis select the fluorescence channel Cycling Green for the analytical BK Virus PCR and the fluorescence channel Cycling Orange 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 "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 BK Virus 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 or urine sample subjected to purification in fluorescence channel Cycling Orange and simultaneous absence of a signal in channel Cycling Green

 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

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 "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 BK Virus 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 of the analytical PCR

 a) Contamination occurred during preparation of the PCR Repeat the PCR with new reagents in replicates.

If possible, close the PCR tubes directly after addition of the sample to be tested.

Make sure to pipet the positive controls last.

Make sure that work space and instruments are decontaminated at regular intervals.

b) Contamination occurred during extraction

Repeat the extraction and PCR of the sample to be tested using new reagents.

Make sure that work space and instruments are decontaminated at regular intervals.

References

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.
artus BK Virus RG PCR Kit (96)	For 96 reactions: Master, Mg Solution, 4 Quantitation Standards, Internal Control, Water (PCR grade)	4514205
purification of viral DN	or automated, simultaneous NA and RNA from 1–6 human plasma, s using the EZ1 Advanced	
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
EZ1 Advanced DSP Virus Card	Preprogrammed card for EZ1 DSP Virus protocol	9018306
EZ1 Advanced	Robotic workstation for automated purification of nucleic acids using EZ1 DSP Kits, 1-year warranty on parts and labor	9001411
Rotor-Gene Q and acc	essories	
Rotor-Gene Q 5plex	Real-time PCR cycler 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
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

Product	Contents	Cat. no.
Loading Block 96 x 0.2 ml Tubes	Aluminum block for manual reaction set-up in a standard 8 x 12 array using 96 x 0.2 ml tubes	9018905
Strip Tubes and Caps, 0.1 ml (250)	250 strips of 4 tubes and caps for 1000 reactions	981103
Strip Tubes and Caps, 0.1 ml (2500)	10 x 250 strips of 4 tubes and caps for 10,000 reactions	981106
PCR Tubes, 0.2 ml (1000)	1000 thin-walled tubes for 1000 reactions	981005
PCR Tubes, 0.2 ml (10000)	10 x 1000 thin-walled tubes for 1000 reactions	981008

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Brazil - Orders 0800-557779 - Fax 55-11-5079-4001 - Technical 0800-557779

Canada = Orders 800-572-9613 = Fax 800-713-5951 = Technical 800-DNA-PREP (800-362-7737)

China = Orders 021-3865-3865 = Fax 021-3865-3965 = Technical 800-988-0325

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The Netherlands = Orders 0800-0229592 = Fax 0800-0229593 = Technical 0800-0229602

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