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artus® BK Virus QS-RGQ Kit Handbook

24

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

For use with QIAsymphony® SP/AS and Rotor-Gene® Q Instruments

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QIAGEN Sample and Assay Technologies

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Contents

Kit Contents

***artus* BK Virus QS-RGQ Kit**			**(24)**
Catalog no.			**4514303**
Number of reactions			**24**
Blue	BK Virus RG Master		4 x 84 µl
Yellow	BK Virus RG Mg-Sol*	**Mg-Sol**	400 µl
Red	BK Virus RG QS 1† (1 x 10^4 copies/µl)	**QS**	200 µl
Red	BK Virus RG QS 2† (1 x 10^3 copies/µl)	**QS**	200 µl
Red	BK Virus RG QS 3† (1 x 10^2 copies/µl)	**QS**	200 µl
Red	BK Virus RG QS 4† (1 x 10^1 copies/µl)	**QS**	200 µl
Green	BK Virus RG IC‡	**IC**	1000 µl
White	Water (PCR grade)		1000 µl
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* Magnesium solution.

† Quantitation standard.

‡ Internal control.

Symbols

<N> Contains reagents sufficient for <N> tests


Use by

Catalog number

Lot number

Material number

Components

Contains

Number

Temperature limitations

Legal manufacturer

Consult instructions for use

Storage

The components of the *artus* BK Virus QS-RGQ 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 performance. If the reagents are to be used only intermittently, they should be frozen in aliquots. Storage at 2–8°C should not exceed a period of 5 hours.

Intended Use

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.

For safety information for the QIAsymphony DSP Virus/Pathogen Midi Kit, see the *QIAsymphony DSP Virus/Pathogen Handbook*. For safety information regarding instruments, see the relevant instrument user manual.

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* BK Virus QS-RGQ Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The *artus* BK Virus QS-RGQ Kit constitutes a ready-to-use system for the detection of BK virus DNA using polymerase chain reaction (PCR) on Rotor-Gene Q Instruments with sample preparation and assay setup using the QIAsymphony SP/AS 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.

In addition, the *artus* BK Virus QS-RGQ 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. 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 20.

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.*

Workflow

The QIAsymphony RGQ workflow starts with purification of nucleic acids from human plasma or urine samples on the QIAsymphony SP instrument. The eluates, containing purified nucleic acids, from the sample preparation procedure are transferred to the QIAsymphony AS module, which then performs assay setup (Figure 1). The assays are then transferred to the Rotor-Gene Q for PCR and data analysis (Figure 2).

A workflow overview is given on pages 9–11. For more information, refer to the detailed protocols in this handbook (pages 21, 28, and 33).

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

Figure 1. Walkaway automation on QIAsymphony SP/AS instruments. Samples, reagents, and consumables are loaded in the relevant drawers. Sample preparation and assay setup are then fully automated on the QIAsymphony SP/AS instruments.

Figure 2. Transferring the samples to the Rotor-Gene Q. After sample preparation and assay setup, the reactions are loaded in the 72-Well Rotor of the Rotor-Gene Q for real-time PCR analysis.

QIAsymphony RGQ workflow overview

QIAsymphony SP

Load the "Waste" drawer.

↓

Place the elution rack in the "Eluate" drawer.

Independent operation	Integrated operation
Place the elution rack on slot 1.	Place the elution rack with adapter, including the transfer frame on slot 1.

↓

Load the "Reagents and Consumables" drawer.

↓

Load the "Sample" drawer.

↓

Define a run/batch using the sample preparation user interface.

↓

Start the protocol.

↓

When sample processing is complete either manually remove the elution rack from the "Eluate" drawer (independent operation), or perform direct transfer of the elution rack to the QIAsymphony AS via the transfer module (integrated operation).

Independent operation	Integrated operation
Remove the eluate slot assignment on the touchscreen, then open the "Eluate" drawer and manually remove the elution rack.	Press "Transfer" to transfer the elution rack from the QIAsymphony SP to the QIAsymphony AS.

↓

Download the QIAsymphony SP result file.

↓

QIAsymphony AS

Switch to the assay setup user interface in the touchscreen.

↓

Start the assay definition process.

Independent operation	Integrated operation
Press "New".	A message will appear asking whether to use the elution rack as a sample rack. Press "Yes" to confirm.

↓

Define an assay run.

The screens that appear on the touchscreen will guide you step-by-step through the assay definition process.

↓

Load the "Eluate and Reagents" and "Assays" drawer with the appropriate samples, reagents, and consumables.

Note: For detailed loading information, refer to the "Loading Information" screen. Alternatively, press "Queue" and download the loading information file.

↓

Start the assay run.

↓

After the run is finished, remove the assays. To do this, press "Remove" and then manually remove the assay rack(s) from the "Assays" drawer.

↓

Transfer the assay rack(s) to the Rotor-Gene Q.

↓

Download the QIAsymphony AS result file and the cycler file.

↓

Transfer the cycler file to the Rotor-Gene Q.

↓

Rotor-Gene Q

Select or create a temperature profile.

↓

Click "Start Run".

↓

After the run is finished, analyze the results.

↓

Maintenance

Perform the appropriate maintenance procedures for the QIAsymphony SP/AS and the Rotor-Gene Q according to the instrument user manuals.

See Section 8 of the *QIAsymphony SP/AS User Manual — General Description* and Section 9 of the *Rotor-Gene Q User Manual* for more details about required daily, regular, and weekly maintenance procedures.

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.

- Pipets (adjustable)* and sterile pipet tips with filters
- Vortex mixer*
- Benchtop centrifuge* with rotor for 2 ml reaction tubes, capable of centrifugation at 6800 x *g*

For sample preparation (plasma or urine)

- QIAsymphony SP instrument (cat. no. 9001297)*
- QIAsymphony DSP Virus/Pathogen Midi Kit (cat. no. 937055)
- Adapters for the QIAsymphony SP:
  - Elution Microtube Rack QS (Cooling Adapter, EMT, v2, Qsym, cat. no. 920730)
  - Tube Insert 3B (Insert, 2.0ml v2, samplecarr. (24), Qsym, cat. no. 9242083)
- Consumables for the QIAsymphony SP:
  - Sample Prep Cartridges, 8-well (cat. no. 997002)
  - 8-Rod Covers (cat. no. 997004)
  - Filter-Tips, 1500 µl (cat. no. 997024)
  - Filter-Tips, 200 µl (cat. no. 990332)
  - Elution Microtubes CL (cat. no. 19588)
  - Tip disposal bags (cat. no. 9013395)
  - Micro tubes 2.0 ml Type H or Micro tubes 2.0 ml Type I (Sarstedt, cat. nos. 72.693 and 72.694, www.sarstedt.com) for use with samples and internal controls

For sample preparation (urine)

- Buffer ATL (cat. no. 939011)

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

For assay setup

- QIAsymphony AS instrument (cat. no. 9001301)*
- Adapters and reagent holders for the QIAsymphony AS:
  - Reagent holder 1 QS (Cooling Adapter, Reagent Holder 1, Qsym, cat. no. 9018090)
  - Reagent holder 2 QS (Cooling Adapter, Reagent Holder 2, Qsym, cat. no. 9018089)
  - RG Strip Tubes 72 QS (Cooling Adapter, RG Strip Tubes 72, Qsym, cat. no. 9018092)
- Consumables for the QIAsymphony AS:
  - Strip Tubes and Caps, 0.1 ml (cat. no. 981103)
  - Tubes, conical, 2 ml, Qsym AS (cat. no. 997102)† or Micro tubes 2.0 ml Type I (Sarstedt, cat. no. 72.694.005, www.sarstedt.com)
  - Tube, conical, 5 ml, Qsym AS (cat. no. 997104)† or Tubes with flat base from PP (Sarstedt, cat. no. 60.558.001, www.sarstedt.com)
  - Reagent Bottles, 30 ml, Qsym AS (cat. no. 997108)
  - Elution Microtubes CL (cat. no. 19588)
  - Filter-Tips, 1500 µl (cat. no. 997024)
  - Filter-Tips, 200 µl (cat. no. 990332)
  - Filter-Tips, 50 µl (cat. no. 997120)
  - Tip disposal bags (cat. no. 9013395)

For PCR

- Rotor-Gene Q 5plex HRM instrument*
- Rotor-Gene Q software version 2.02, or higher

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

† Please inquire for availability.

Important Notes

General precautions

Always pay attention to the following:

- Use sterile pipet tips with filters.
- During manual steps, keep tubes closed when possible and avoid contamination.
- 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. Ensure that no foam or bubbles are present in the reagent tubes.
- Do not mix components from kits with different lot numbers.
- Make sure that the required adapters are precooled to 2–8°C.
- Work quickly and keep PCR reagents on ice or in the cooling block before loading.
- Proceed continuously from one part of the workflow to the next. Do not exceed one hour of transfer time between each module (QIAsymphony SP to QIAsymphony AS to Rotor-Gene Q).

Getting started on the QIAsymphony SP/AS instruments

Close all drawers and the hoods.

Switch on the QIAsymphony SP/AS instruments, and wait until the "Sample Preparation" screen appears and the initialization procedure has finished.

Log into the instrument (drawers will unlock).

Loading reagent cartridges (RC) into the "Reagents and Consumables" drawer

Reagents for purification of nucleic acids are contained in an innovative reagent cartridge (RC) (see Figure 3). Each trough of the reagent cartridge (RC) contains a particular reagent, such as magnetic particles, lysis buffer, wash buffer, or elution buffer. Partially used reagent cartridges (RC) can be reclosed with Reuse Seal Strips for later reuse, which avoids generation of waste due to leftover reagents at the end of the purification procedure.

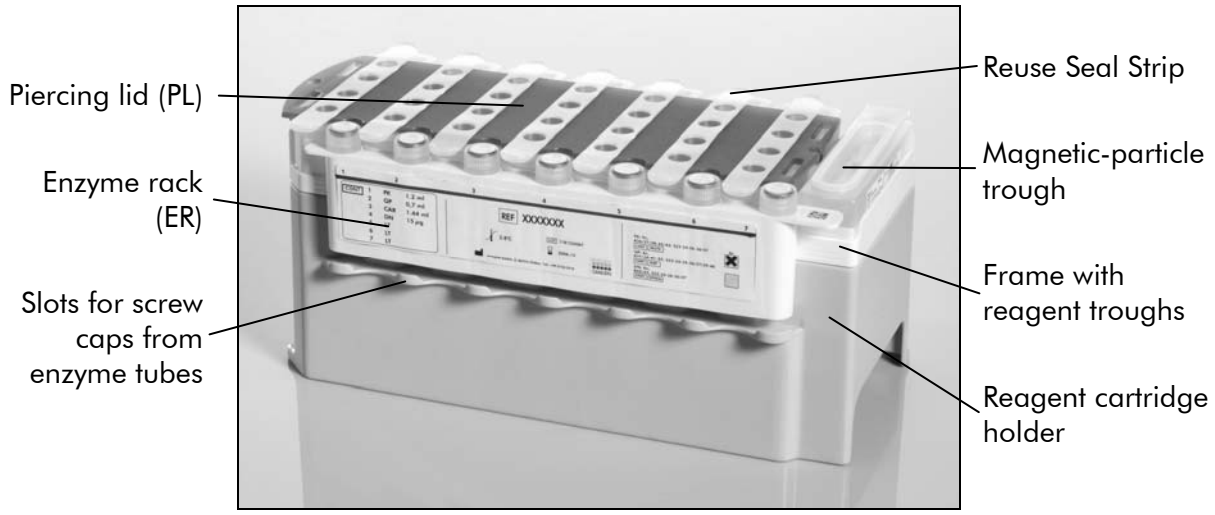

Figure 3. QIAsymphony reagent cartridge (RC). The reagent cartridge (RC) contains all reagents required for the purification procedure.

Before starting the procedure, ensure that the magnetic particles are fully resuspended. Remove the magnetic-particle trough from the reagent cartridge frame, vortex it vigorously for at least 3 minutes, and replace it in the reagent cartridge frame before the first use. Place the reagent cartridge (RC) into the reagent cartridge holder. Place the enzyme rack (ER) into the reagent cartridge holder. Before using a reagent cartridge (RC) for the first time, place the piercing lid (PL) on top of the reagent cartridge (RC) (Figure 4).

Note: The piercing lid (PL) is sharp. Take care when placing it onto the reagent cartridge (RC). Make sure to place the piercing lid (PL) onto the reagent cartridge (RC) in the correct orientation.

After the magnetic-particle trough cover is removed and the enzyme rack tubes are opened (screw caps can be stored in dedicated slots, see Figure 3), the reagent cartridge (RC) is subsequently loaded into the "Reagents and Consumables" drawer.

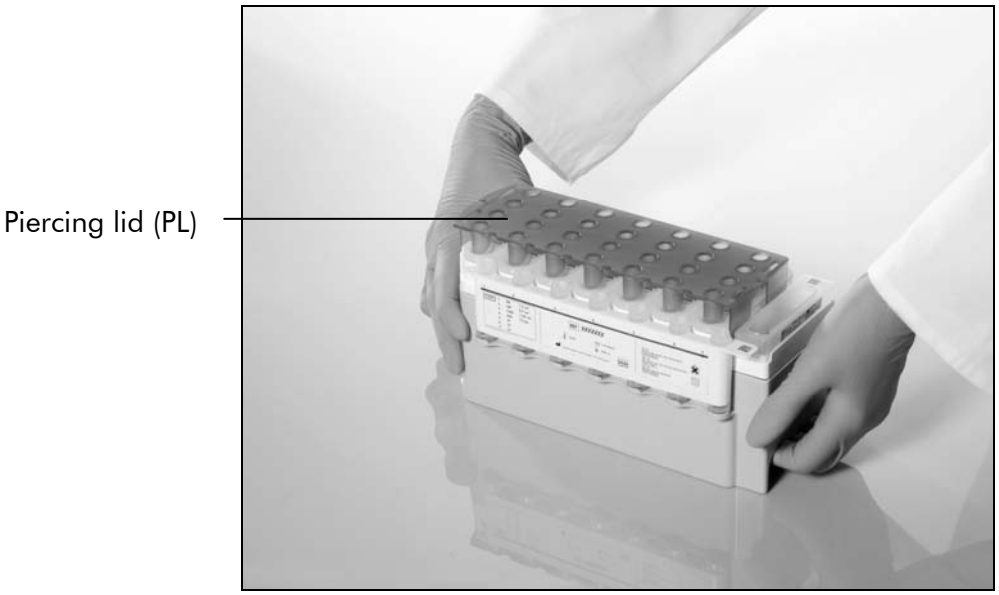

Figure 4. Easy worktable setup with reagent cartridges (RC).

Partially used reagent cartridges (RC) can be stored until needed again, see "Storage" in the *QIAsymphony DSP Virus/Pathogen Handbook*.

Loading plasticware into the "Reagents and Consumables" drawer

Sample prep cartridges, 8-Rod Covers (both preracked in unit boxes), and disposable filter-tips (200 µl tips provided in blue racks, 1500 µl tips provided in black racks) are loaded into the "Reagents and Consumables" drawer.

Note: Ensure that the covers of the unit boxes are removed before loading the unit boxes into the "Reagents and Consumables" drawer.

Note: Tips have filters to help prevent cross-contamination.

Tip rack slots on the QIAsymphony SP worktable can be filled with either type of tip rack. The QIAsymphony SP will identify the type of tips loaded during the inventory scan.

Note: Do not refill tip racks or unit boxes for sample prep cartridges or 8-Rod Covers manually before starting another protocol run. The QIAsymphony SP can use partially used tip racks and unit boxes.

Loading the "Waste" drawer

Sample prep cartridges and 8-Rod Covers used during a run are re-racked in empty unit boxes in the "Waste" drawer. Make sure that the "Waste" drawer contains sufficient empty unit boxes for plastic waste generated during the protocol run.

Note: Ensure that the covers of the unit boxes are removed before loading the unit boxes into the "Waste" drawer. If you are using 8-Rod Cover boxes for collecting used sample prep cartridges and 8-Rod Covers, ensure that the box spacer has been removed.

A bag for used filter-tips must be attached to the front side of the "Waste" drawer.

Note: The presence of a tip disposal bag is not checked by the system. Make sure that the tip disposal bag is properly attached before starting a protocol run. For more information, see the user manuals provided with your instrument. Empty the tip bag after a maximum of 96 samples have been processed to avoid a tip jam.

A waste container collects liquid waste generated during the purification procedure. The "Waste" drawer can only be closed if the waste container is in place. Dispose of the liquid waste according to your local safety and environment regulations. Do not autoclave the filled waste bottle. Empty the waste bottle after a maximum of 96 samples have been processed.

Loading the "Eluate" drawer

Load the required elution rack into the "Eluate" drawer. Use "Elution slot 1" with the corresponding cooling adapter. For integrated operation, place the elution rack with adapter, including the transfer frame on slot 1.

Inventory scan

Before starting a run, the instrument checks that sufficient consumables for the queued batch(es) have been loaded into the corresponding drawers.

Preparation of sample material

The QIAsymphony DSP Virus/Pathogen Midi Kit is suitable for use with human plasma or urine samples. Prevent formation of foam in or on the samples. Samples should be equilibrated to room temperature (15–25°C) before starting the run.

Preparing carrier RNA (CARRIER)–Buffer AVE (AVE) mixtures

Note: Carrier RNA (CARRIER) must be used.

To prepare a carrier RNA (CARRIER) stock solution, add 1350 µl Buffer AVE (AVE) (provided in 2 ml vials) to the tube containing 1350 µg lyophilized carrier RNA (CARRIER) to obtain a solution of 1 µg/µl. Dissolve the carrier RNA (CARRIER) thoroughly, divide it into conveniently sized aliquots, and store at 2–8°C for up to 2 weeks.

Calculating the volume of carrier RNA (CARRIER) mixture per tube

The minimum volume of carrier RNA (CARRIER)–Buffer AVE (AVE) mixture must include sufficient additional volume to take into account liquid loss due to pipetting and evaporation. See Table 1 for volumes to use for analysis with the *artus* BK Virus QS-RGQ Kit.

Tubes containing carrier RNA (CARRIER)–Buffer AVE (AVE) and internal control mixtures are placed in a tube carrier. The tube carrier containing the carrier RNA (CARRIER)–Buffer AVE (AVE) mixture(s) must be placed in slot A of the sample drawer. Up to 8 tubes of the mixture can be used per batch and up to 24 tubes can be used per run of 4 batches.

Using an internal control

An internal control (BK Virus RG IC) is supplied with the *artus* BK Virus QS-RGQ Kit. This allows the user both to control the DNA isolation procedure and to check for possible PCR inhibition.

Using the QIAsymphony DSP Virus/Pathogen Midi Kit in combination with the *artus* BK Virus QS-RGQ Kit requires introduction of the internal control (BK Virus RG IC) into the purification procedure to monitor the efficiency of sample preparation and downstream assay.

Internal controls must be added with carrier RNA (CARRIER)–Buffer AVE (AVE) mixture, and the total volume of the internal control–carrier RNA (CARRIER)–Buffer AVE (AVE) mixture remains 120 µl.

For this application, add the internal control to the isolation as shown in Table 1. This represents addition of internal control to the isolation at a ratio of 0.1 µl per 1 µl elution volume. We recommend preparing fresh mixtures for each run just before use.

Table 1. Preparation of internal control–carrier RNA (CARRIER)

Component	Plasma samples Volume (µl)	Urine samples Volume (µl)
Stock carrier RNA (CARRIER)	5	3
Internal control*	9	9
Buffer AVE	106	108
Final volume per sample (excluding dead volume)	**120**	**120**
Total volume for n samples†	**(n x 120) + 360‡**	**(n x 120) + 360‡**

* The calculation of the amount of internal control is based on the initial elution volumes (90 µl). Additional void volume depends on the type of sample tube used.

† Do not fill more than 1.92 ml (corresponding to a maximum of 13 samples; this volume is specific for Micro tubes 2.0 ml Type H and Micro tubes 2.0 ml Type I, Sarstedt cat. nos. 72.693 and 72.694).

‡ Internal control mixture corresponding to 3 additional samples (i.e., 360 µl) is required (this volume is specific for Micro tubes 2.0 ml Type H and Micro tubes 2.0 ml Type I, Sarstedt cat. nos. 72.693 and 72.694).

Assay Control Sets and Assay Parameter Sets

Assay Control Sets are the combination of a protocol plus additional parameters, such as internal control, for sample purification on the QIAsymphony SP. A default Assay Control Set is preinstalled for each protocol.

Assay Parameter Sets are the combination of an assay definition with additional parameters defined, such as replicate count and number of assay standards, for assay setup on the QIAsymphony AS.

Yields of nucleic acids

Eluates prepared with carrier RNA (CARRIER) may contain much more carrier RNA (CARRIER) than target nucleic acids. We recommend using quantitative amplification methods to determine yields.

Storing nucleic acids

For short-term storage of up to 24 hours, we recommend storing purified nucleic acids at 2–8°C. For long-term storage of over 24 hours, we recommend storage at –20°C.

Setting the threshold for the PCR analysis

The optimal threshold settings for a given combination of Rotor-Gene Q instrument and *artus* QS-RGQ Kit should be set empirically by testing each individual combination since it is a relative value depending on the overall diagnostic workflow. As a starting point, the threshold can be set at a preliminary value of 0.04 for the analysis of the first PCR run, but this value should be fine-tuned in a comparative analysis of the next runs of the workflow. The threshold should be set manually just above the background signal of the negative controls and negative samples. The mean threshold value calculated from these experiments will most likely work for the majority of future runs, but the user should nevertheless review the generated threshold value at regular intervals. The threshold value will usually be in the range of 0.03–0.05 and should be rounded to no more than three decimal places.

Quantitation

The quantitation standards (BK Virus RG QS 1–4) in the *artus* BK Virus QS-RGQ Kit are treated as previously purified samples and the same volume is used (15 µl). To generate a standard curve on Rotor-Gene Q Instruments, all 4 quantitation standards should be used and defined in the "Edit Samples" dialog box on the Rotor-Gene Q instrument as standards with the specified concentrations (see the instrument user manual).

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

$$\text{Result (copies/ml)} = \frac{\text{Result (copies/µl) x Initial Elution Volume (90 µl)*}}{\text{Sample Volume (ml)}}$$

As a matter of principle the initial sample volume should be entered in the equation above. This has to be considered when the sample volume has been changed prior to the nucleic acid extraction (e.g., reducing the volume by centrifugation or increasing the volume by adding to the volume required for the isolation).

* The calculation is based on the initial elution volumes (90 µl).

Protocol: DNA Isolation on the QIAsymphony SP

The QIAsymphony DSP Virus/Pathogen Midi Kit (QIAGEN, cat. no. 937055) may be used for viral DNA purification from human EDTA plasma or urine for use with the *artus* BK Virus QS-RGQ Kit. General information about the protocol is shown in Table 2.

Table 2. General protocol information

Kit	QIAsymphony DSP Virus/Pathogen Midi Kit	QIAsymphony DSP Virus/Pathogen Midi Kit
Sample material	Plasma	Urine
Sample volume (including excess volume)	1200 µl	1000 µl
Protocol name	Cellfree1000_V4_DSP*	Complex800_V5_DSP†
Default Assay Control Set	Cellfree1000_V4_DSP artus BKV*	Complex800_V5_DSP artus BKV†
Elution volume	60 µl	60 µl
Required software version	Version 3.1 or higher	Version 3.5 or higher

* V4 or higher, as required by the software version used.

† V5 or higher, as required by the software version used.

Important points before starting

- Ensure that you are familiar with operating the QIAsymphony SP/AS instruments. Refer to the user manuals supplied with your instruments for operating instructions.
- Before beginning the procedure, read “Important Notes”, pages 14–20.

- Before using a reagent cartridge (RC) for the first time, check that Buffers QSL2 and QSB1 in the cartridge (RC) do not contain a precipitate. If necessary, remove the troughs containing Buffers QSL2 and QSB1 from the reagent cartridge (RC) and incubate for 30 minutes at 37°C with occasional shaking to dissolve precipitate. Make sure to replace the troughs in the correct positions. If the reagent cartridge (RC) is already pierced, make sure that the troughs are sealed with Reuse Seal Strips and incubate the complete reagent cartridge (RC) for 30 minutes at 37°C with occasional shaking in a water bath.*
- Try to avoid vigorous shaking of the reagent cartridge (RC) otherwise foam may be generated, which can lead to liquid-level detection problems.
- All components of the QIAsymphony DSP Virus/Pathogen Midi Kit have been shown to be stable onboard the QIAsymphony SP/AS instruments for at least the normal time required to process 96 samples.

Things to do before starting

- Prepare all required mixtures, including mixtures containing carrier RNA (CARRIER) and internal controls just before starting. For more information, see "Preparing carrier RNA (CARRIER)–Buffer AVE (AVE) mixtures", page 17, and "Using an internal control", page 18.
- Make sure that the piercing lid (PL) is placed on the reagent cartridge (RC) and the lid of the magnetic-particle trough has been removed or, if using a partially used reagent cartridge (RC), make sure the Reuse Seal Strips have been removed.
- Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex the trough containing the magnetic particles vigorously for at least 3 minutes before first use.
- Before loading the reagent cartridge (RC), remove the cover from the trough containing the magnetic particles and open the enzyme tubes. Make sure that the enzyme rack has been equilibrated to room temperature (15–25°C). Make sure that the piercing lid (PL) is placed on the reagent cartridge (RC) or, if using a partially used reagent cartridge, make sure the Reuse Seal Strips have been removed.
- If samples are bar coded, orient samples in the tube carrier so that the bar codes face the bar code reader within the "Sample" drawer at the left side of the QIAsymphony SP.

* Ensure that instruments have been checked, maintained, and calibrated regularly according to the manufacturer's instructions.

Procedure

1. **Close all drawers and the hoods of the QIAsymphony SP/AS instruments.**
2. **Switch on the instruments, and wait until the "Sample Preparation" screen appears and the initialization procedure has finished.**
   The power switch is located at the bottom, left corner of the QIAsymphony SP.
3. **Log in to the instruments.**
4. **Ensure the "Waste" drawer is prepared properly (see Table 3), and perform an inventory scan of the "Waste" drawer, including the tip chute and liquid waste. Replace the tip disposal bag if necessary.**

Table 3. Preparing the "Waste" drawer

Unit box holder 1–4	Empty unit boxes
Waste bag holder	Apply waste bag
Liquid waste bottle holder	Empty and install liquid waste bottle

5. **Load the required elution rack into the "Eluate" drawer.**
   Use only "Elution slot 1" with the corresponding cooling adapter (Cooling Adapter, EMT, v2, Qsym, cat. no. 920730). For integrated operation, place the elution rack with adapter, including the transfer frame on slot 1.
6. **Load the required reagent cartridge(s) (RC) and consumables into the "Reagents and Consumables" drawer (see Tables 4–6).**
   Note: Numbers of filter-tips given may differ from the numbers displayed in the touchscreen depending on settings.

Table 4. Preparing the "Reagents and Consumables" drawer

	Plasma samples	Urine samples
Position A1 and/or A2	Load 1 reagent cartridge (RC) for up to 48 samples or 2 new reagent cartridges (RC) for up to 96 samples	
Position B1	–	Buffer ATL (ATL)
Tip rack holder 1–17	Load sufficient racks of disposable filter-tips, 200 µl and 1500 µl (see Table 5, for plasma, or Table 6, for urine)	
Unit box holder 1–4	Load unit boxes containing sample prep cartridges and 8-Rod Covers (see Table 5, for plasma, or Table 6, for urine)	

Table 5. Required plasticware for 1–4 plasma sample batches

	One batch, 24 samples*	Two batches, 48 samples*	Three batches, 72 samples*	Four batches, 96 samples*
Disposable filter-tips, 200 µl†‡	28	52	76	100
Disposable filter-tips, 1500 µl†‡	113	206	309	402
Sample prep cartridges§	21	42	54	72
8-Rod Covers¶	3	6	9	12

* Use of more than one internal control tube per batch and performing more than one inventory scan requires additional disposable filter tips.

† There are 32 filter-tips/tip rack.

‡ Number of required filter-tips includes filter-tips for 1 inventory scan per reagent cartridge.

§ There are 28 sample prep cartridges/unit box.

¶ There are twelve 8-Rod Covers/unit box.

Table 6. Required plasticware for 1–4 urine sample batches

	One batch, 24 samples*	Two batches, 48 samples*	Three batches, 72 samples*	Four batches, 96 samples*
Disposable filter-tips, 200 µl†‡	34	60	86	112
Disposable filter-tips, 1500 µl†‡	123	205	295	385
Sample prep cartridges§	18	36	54	72
8-Rod Covers¶	3	6	9	12

* Use of more than one internal control tube per batch and performing more than one inventory scan requires additional disposable filter tips.

† There are 32 filter-tips/tip rack.

‡ Number of required filter-tips includes filter-tips for 1 inventory scan per reagent cartridge.

§ There are 28 sample prep cartridges/unit box.

¶ There are twelve 8-Rod Covers/unit box.

7. **Perform an inventory scan of the "Reagents and Consumables" drawer.**
8. **Place the samples into the appropriate tube carrier, and load them into the "Sample" drawer (see Table 7).**

Table 7. Loading samples into the "Sample" drawer

Sample type	Plasma	Urine
Sample volume (including excess volume)	1200 µl	1000 µl
Sample tubes	Micro tubes 2.0 ml Type H or Micro tubes 2.0 ml Type I (Sarstedt, cat. nos. 72.693 and 72.694)	
Insert	Tube Insert 3B (cat. no. 9242083)	

9. **Place the tube(s) containing the carrier RNA (CARRIER)–Buffer AVE (AVE) mixture, including internal control, into the tube carrier and load into slot A of the "Sample" drawer.**

   For more information about preparing the mixture, see "Preparing carrier RNA (CARRIER)–Buffer AVE (AVE) mixtures", page 17, and "Using an internal control", page 18.

10. **Using the touchscreen, enter the required information for each batch of samples to be processed.**

    Enter the following information:

    - Sample information
    - Protocol to be run (choose Assay Control Set "Cellfree1000_V4_DSP artus BKV"* for plasma or "Complex800_V5_DSP_artus BKV"† for urine from category "artus_QS-RGQ")
    - Elution volume 60 µl (see Table 8) and output position "slot 1"
    - Tube(s) containing the carrier RNA (CARRIER)–Buffer AVE (AVE) mixture, including internal control (choose IC name "Cellfree1000_V4_DSP artus BKV"* for plasma or "Complex800_V5_DSP_artus BKV"† for urine)

    After information about the batch has been entered, the status changes from "LOADED" to "QUEUED". As soon as one batch is queued the "Run" button appears.

Table 8. Preselected elution volume

Preselected elution volume (µl)‡	Initial elution volume (µl)§
60	90

‡ The elution volume preselected for the protocol. This is the minimum accessible volume of eluate in the final elution tube.

§ The initial volume of elution solution required to ensure that the actual volume of eluate is the same as the preselected volume.

11. **Press the "Run" button to start the purification procedure.**

    All processing steps are fully automated. At the end of the protocol run, the status of the batch changes from "RUNNING" to "COMPLETED".

* V4 or higher, as required by the software version used.

† V5 or higher, as required by the software version used.

12. **When sample processing is complete, either manually remove the elution rack from the "Eluate" drawer (independent operation), or perform direct transfer of the elution rack to the QIAsymphony AS via the transfer module (integrated operation).**
    For independent operation, remove the elution rack from the slot on the touchscreen, and then open the "Eluate" drawer and manually remove the elution rack.
    For integrated operation, press "Transfer" to transfer the elution rack from slot 1 of the QIAsymphony SP to slot 2 of the QIAsymphony AS.

    We recommend immediately (≤60 min) proceeding with assay setup on the QIAsymphony AS after the run has finished. Depending on temperature and humidity, eluates left in the QIAsymphony SP after the run is completed may experience condensation or evaporation.

    For short-term storage of up to 24 h, we recommend storing purified nucleic acids at 2–8°C. For long-term storage of over 24 h, we recommend storage purified nucleic acids at –20°C.

    Result files are generated for each elution rack.

13. **If a reagent cartridge (RC) is only partially used, seal it with the provided Reuse Seal Strips and close tubes containing proteinase K with screw caps immediately after the end of the protocol run to avoid evaporation.**

    Note: For more information about storage of partially used reagent cartridges (RC), see "Storage" in the *QIAsymphony DSP Virus/Pathogen Handbook*.

14. **Discard used sample tubes, plates, and waste according to your local safety regulations.**

    See the *QIAsymphony DSP Virus/Pathogen Handbook* for safety information.

15. **Close the instrument drawers, and proceed with assay setup on the QIAsymphony AS (page 28).**
16. **Clean the QIAsymphony SP during the assay setup on the QIAsymphony AS or later.**

    For integrated operation, clean all instruments at the end of the completed workflow.

    Follow the maintenance instructions in the *QIAsymphony SP/AS User Manual — General Description*. Make sure to carry out maintenance regularly to minimize the risk of cross-contamination.

Protocol: Assay Setup on the QIAsymphony AS

The QIAsymphony AS carries out assay setup for 6–216 reactions. Samples processed on the QIAsymphony SP can be transferred automatically to the QIAsymphony AS (integrated operation). For extra flexibility, the QIAsymphony SP and AS can also be operated independently.*

Details of the assay are shown in Table 9.

Table 9. Assay parameters using the *artus* BK Virus QS-RGQ Kit

Process information	Details
Sample input material	Eluate
Master mix volume	10 µl
Template volume	15 µl
Number of reactions	6–24†
Runtime‡	For 6 reactions: approximately 8 minutes For 72 reactions: approximately 35 minutes
Required software version	Version 3.1 or higher

† For assay setup for BK virus, up to 216 (9 x 24) assays can be set up in one run on the QIAsymphony AS.

‡ Assays were run with each reaction unique (no replicates), and master mix was prepared by the instrument.

Important points before starting

- Before beginning the procedure, read "Important Notes", pages 14–20.
- The reagent volumes are optimized for 24 reactions per kit per run.
- Before each use, all reagents need to be thawed completely, mixed (by repeated up and down pipetting or by quick vortexing), and centrifuged for at least 3 s at 6800 x *g*. Avoid foaming of the reagents.
- Eluates from the sample preparation and all components of *artus* QS-RGQ Kits have been shown to be stable onboard the instrument with selected *artus* QS-RGQ systems for at least the normal time required for sample purification for 96 samples and assay setup of 216 assays, including up to 60 min transfer time from the QIAsymphony SP to the QIAsymphony AS and up to 60 min transfer time from the QIAsymphony AS to the Rotor-Gene Q.

* The QIAsymphony SP and AS remain physically connected during independent operation.

- Work quickly and keep PCR reagents on ice or in the cooling block before loading.

Procedure

1. **Insert the tip chute.**
2. **Discard the tip disposal bag.**
3. **Install an empty tip disposal bag.**
4. **Switch to the assay setup user interface by pressing the "Switch" button in the status bar.**
5. **Start the assay definition process.**
   For independent operation, press "New".
   For integrated operation, a message will appear asking whether to use the elution rack as a sample rack. Press "Yes" to confirm.
   The screens on the touchscreen guide the user through the assay definition process in a step-by-step fashion.
6. **Assign the sample rack to a "Sample" slot by selecting the rack file from the respective run on the QIAsymphony SP.**
   Note: If the elution rack has been automatically transferred to the QIAsymphony AS, the rack file will be automatically assigned and the "Sample Rack(s)" screen of the assay setup user interface will already be filled out.
7. **Check sample rack for sample, EC+ and EC– positions, sample IDs, and volumes.**
8. **Select the Assay Parameter Set to use in the run (see Table 10).**

Table 10. Assay information using the *artus* BK Virus QS-RGQ Kit

Release information	Details
Category	artus QS-RGQ
Assay Parameter Set	artus BKV QS-RGQ V1*

* V1 or higher, as required by the software version used.

9. **Assign the Assay Parameter Set to samples.**
   The Assay Parameter Set defines the parameters of the assay setup.
10. **Define assay rack(s). At this stage, assay rack(s) can be loaded onto the "Assay" slot(s) (see Table 12).**
11. **Check the temperature of the cooling positions.**
    When the target cooling temperatures are reached, the slots will appear green.

12. **Combine all tubes of BK Virus RG Master in a single kit into one tube.**

    Note: Viscous reagents can be difficult to handle with manual pipets. Make sure to transfer the entire volume of BK Virus RG Master in the tube.

13. **Fill each reagent tube with the required volume of appropriate reagent according to the loading information given by the instrument software.**

    Note: Before each use, all reagents need to be thawed completely, mixed (by repeated up and down pipetting or by quick vortexing), and centrifuged for at least 3 s at 6800 x g. Avoid bubbles or foaming, which could cause detection errors. Work quickly and keep PCR components on ice or in the cooling block before loading.

14. **Place the reagent tubes, without lids, into the appropriate positions of precooled adapters for reagents (see Table 11).**

Table 11. Consumables

Consumables	Name on touchscreen	For use with adapter/reagent holder
Strip Tubes and Caps, 0.1 ml (250)	QIA#981103 *StripTubes 0.1	RG Strip Tubes 72 QS
Tubes, conical, 2 ml, Qsym AS (500)*†	QIA#997102 *T2.0 ScrewSkirt‡	Reagent holder 1 QS Reagent holder 2 QS
Tube, conical, 5 ml, Qsym AS (500)*†	QIA#997104 *T5.0 ScrewSkirt‡	Reagent holder 1 QS Reagent holder 2 QS
Reagent Bottles, 30ml, Qsym AS (50)*	QIA#997108 *Bottle 30ml‡	Reagent holder 2 QS
Elution Microtubes CL (24 x 96)	QIA#19588 * EMTR	Elution Microtube Rack QS

* For master mix components, system-prepared master mix, assay standards, and assay controls.

† Alternatively, the Sarstedt tubes described on page 13 can be used.

‡ The suffix "(m)" in the touchscreen indicates that liquid level calculations for the respective tube have been optimized for reagents forming a concave meniscus.

15. **Open the "Eluate and Reagents" and "Assays" drawers.**

16. **Load the prepared reagent adapter into the appropriate slot. Enter a kit bar code for the assay through the "List view" of the "Loading Reagents" screen.**
17. **If not already done, place the Strip Tubes into the precooled RG Strip Tubes 72 QS adapter (see Table 12), and place into the appropriate slot(s).**

Table 12. Adapters and reagent holders

Rack/reagent holder	Name	Number required*
Sample rack	Elution Microtube Rack QS	1
Reagent holders	Reagent holder 1 QS	1
Assay racks	RG Strip Tubes 72 QS	1

* Calculated for an assay run with 72 reactions.

18. **If the elution rack has not already been transferred to the QIAsymphony AS (see step 12 of the purification protocol, page 26), place the Elution Microtubes CL into the precooled Elution Microtube Rack QS adapter (see Table 12), and place the prepared adapter into the slot indicated.**
    For independent operation, remove the elution rack from the slot on the touchscreen, and then open the "Eluate" drawer of the QIAsymphony SP and manually remove the elution rack.
19. **Load disposable filter-tips into the "Eluate and Reagents" and "Assays" drawers, according to the required number of each tip type (see Tables 13 and 14).**

    Load tip racks starting with tip slots 1, 2, and 3 in the "Eluate and Reagents" drawer, and then load tip racks into tip slots 7, 8, and 9 in the "Assays" drawer.

Table 13. Filter-tips

Consumables	Name on touchscreen
Filter-Tips, 1500 µl (1024)	1500 µl
Filter-Tips, 200 µl (1024)	200 µl
Filter-Tips, 50 µl (1024)	50 µl

Table 14. Consumables consumption*

Consumable	Number for 24 reactions	Number for 72 reactions
Filter-Tips, 1500 µl	3	4
Filter-Tips, 200 µl	5	5
Filter-Tips, 50 µl	25	73
Tip Disposal Bags	1	1

* This table provides example consumables consumption for an assay run with the following specifications: 19 or 67 templates, 4 assay standards, one no template control (NTC), samples with internal control (IC), master mix prepared by the instrument.
Note: Filter-tip consumption can be affected by some processes on the QIAsymphony AS (e.g., liquid-level detection).

20. **Close the "Eluate and Reagents" and "Assays" drawers.**
21. **Upon closing each drawer, press "Yes" to start the inventory scan for each drawer.**

    The inventory scan checks the slots, adapters, filter-tips, and the tip chute. If required, correct any errors.
22. **Press "Queue". Monitoring of the cooling starts.**
23. **Wait until the target cooling temperatures are reached.**

    When the target cooling temperatures are reached, the slots will appear green.
24. **Press "Run" to start the run.**
25. **After the run is finished, press "Remove" in the assay setup "Overview" screen. Open the "Assays" drawer and unload the assay rack(s).**
26. **Download the result and cycler files.**
27. **Proceed to "Protocol: PCR and Data Analysis on the Rotor-Gene Q", page 33.**
28. **Perform the regular maintenance of the QIAsymphony AS during the PCR run on the Rotor-Gene Q or later.**

    For integrated operation, clean all instruments at the end of the completed workflow.

    Follow the maintenance instructions in the *QIAsymphony SP/AS User Manual — General Description*. Make sure to carry out maintenance regularly to minimize the risk of cross-contamination.

Protocol: PCR and Data Analysis on the Rotor-Gene Q

Important points before starting

- Before beginning the procedure, read "Important Notes", pages 14–20.
- Take time to familiarize yourself with the Rotor-Gene Q before starting the protocol. See the instrument user manual.
- Make sure that all 4 quantitation standards as well as at least 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.

Procedure

1. **Close the PCR tubes, and place them in the 72-Well Rotor of the Rotor-Gene Q. Make sure to transfer the Rotor-Gene Q 4-strip tubes in the correct orientation, so that the position indices of the cooling adapter and the rotor match. 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.**
2. **Transfer the cycler file from the QIAsymphony AS to the Rotor-Gene Q computer.**
3. **For the detection of BK virus DNA, create a temperature profile according to the following steps.**

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

All specifications refer to the Rotor-Gene Q software version 2.02. Please find further information on programming Rotor-Gene Q instruments in the instrument user manual. In the illustrations these settings are framed in bold black.

4. **First, open the "New Run Wizard" dialog box (Figure 5). Check the "Locking Ring Attached" box and click "Next".**

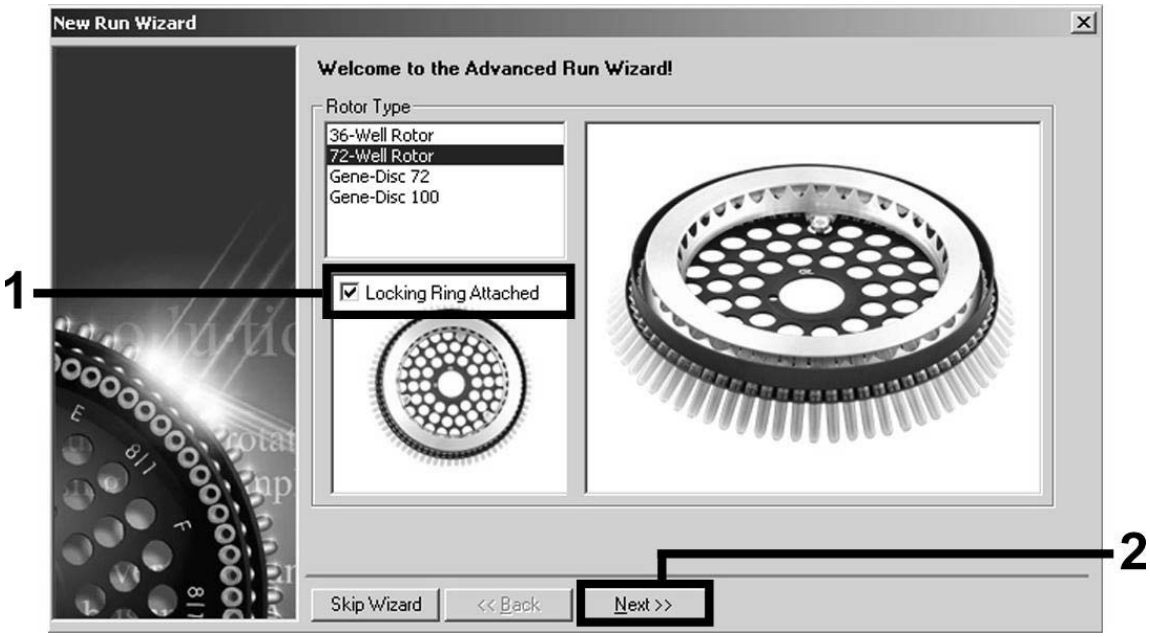

Figure 5. The "New Run Wizard" dialog box.

5. **Select *50* for the PCR reaction volume and click "Next" (Figure 6).**

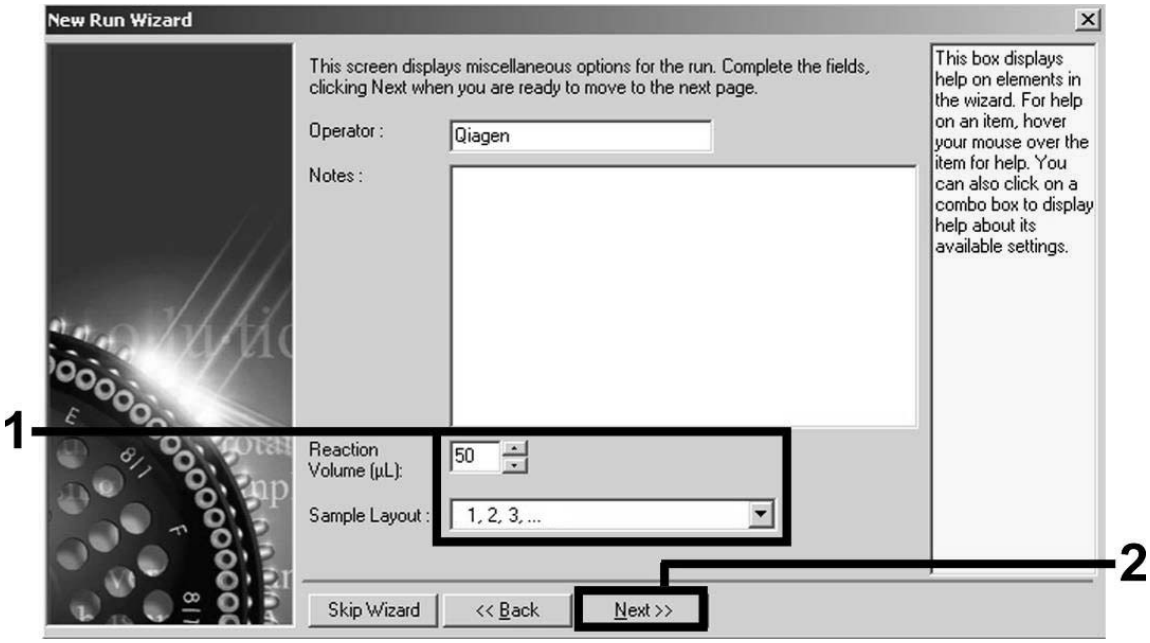

Figure 6. Setting the general assay parameters.

Note: Although the physical reaction volume is 25 µl, make sure to select *50* for the reaction volume in the Rotor-Gene software.

6. **Click the "Edit Profile" button in the next "New Run Wizard" dialog box (Figure 7), and program the temperature profile as shown in Figures 7–9).**

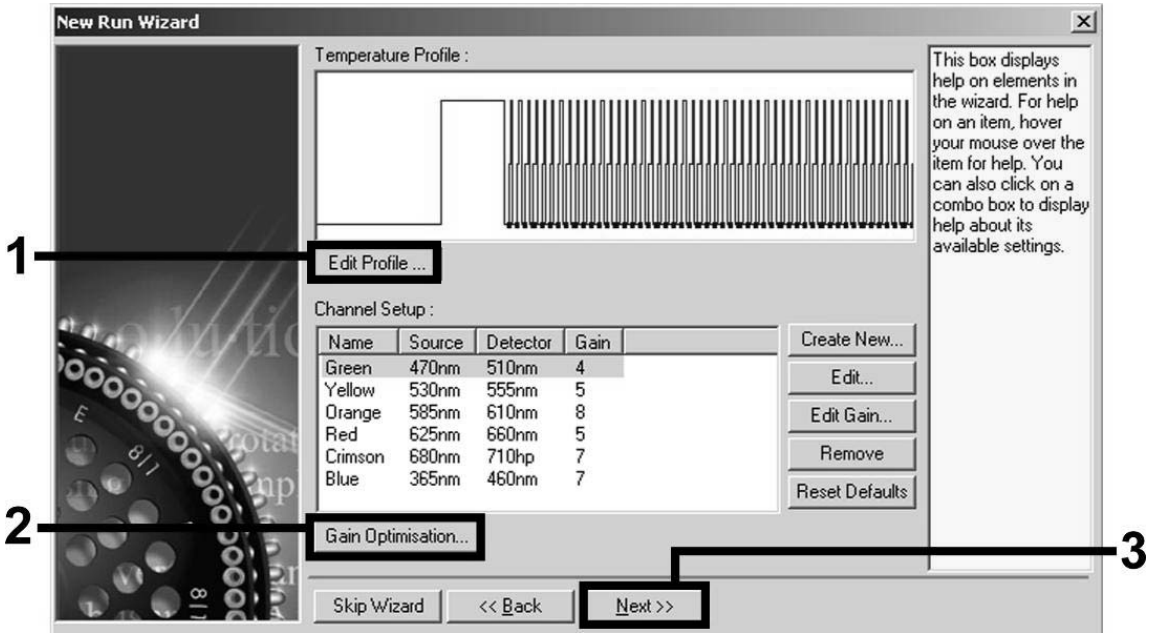

Figure 7. Editing the profile.

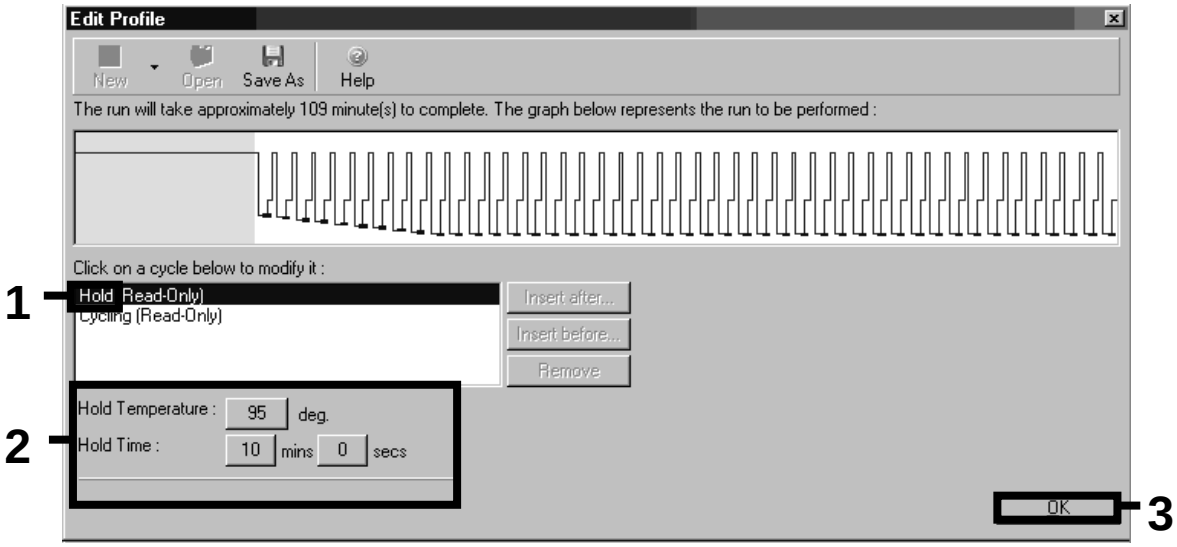

Figure 8. Initial activation of the hot-start enzyme.

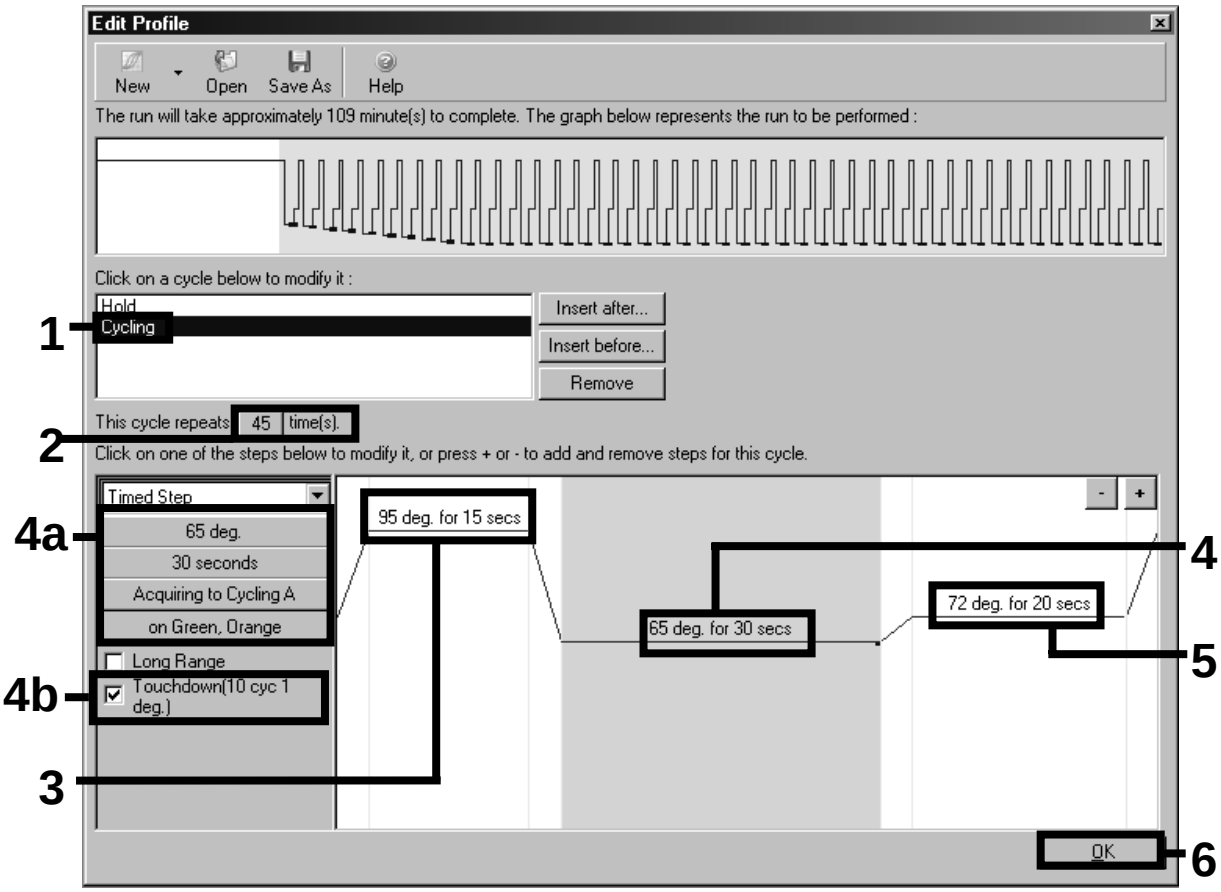

Figure 9. Amplification of the DNA. Make sure to activate the touchdown function for 10 cycles in the Annealing step.

7. **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 7) 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 10).**

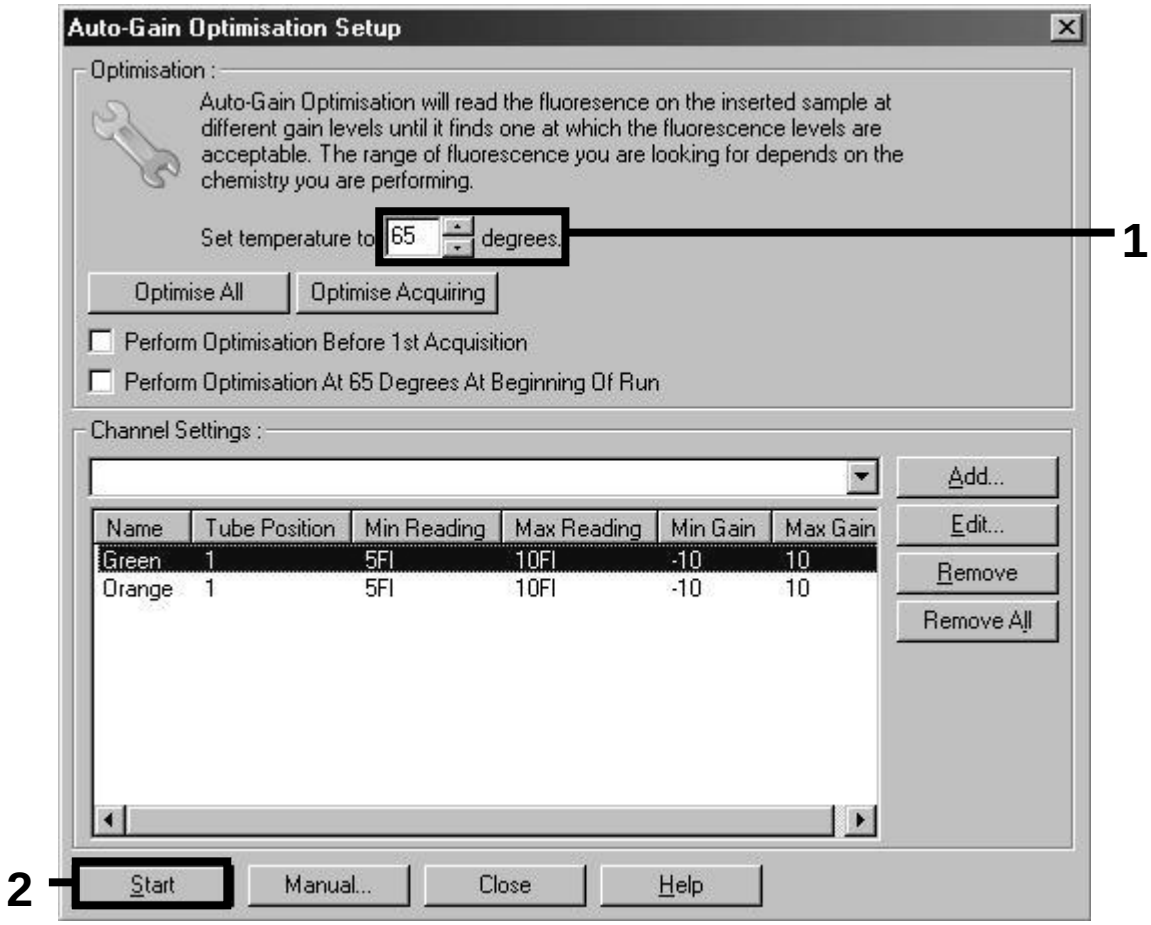

Figure 10. Adjusting the fluorescence channel sensitivity.

8. **The gain values determined by the channel calibration are saved automatically and are listed in the last menu window of the programming procedure (Figure 11). Click "Start Run".**

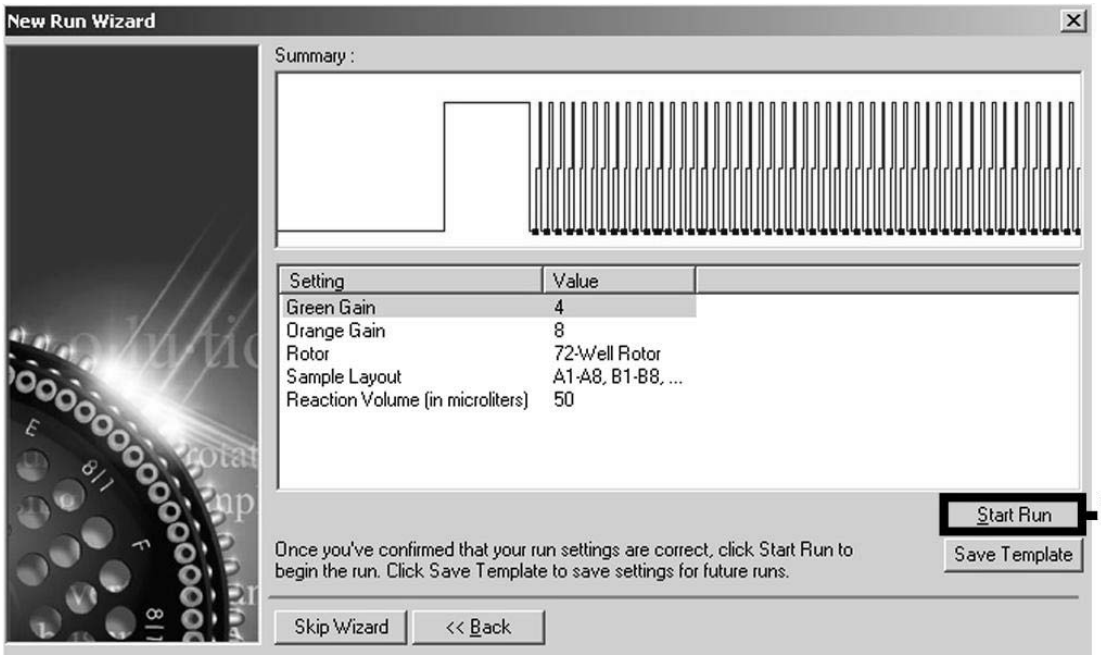

Figure 11. Starting the run.

9. **After starting the run, import the information from the cycler file by clicking the  ("Open") button, or edit the samples manually.**
10. **After the run is finished, analyze the data. The following results (10a, 10b, and 10c) are possible.**

    Examples of positive and negative PCR reactions are given in Figure 12 and Figure 13.

10a. 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).

10b. 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.

10c. 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 40.

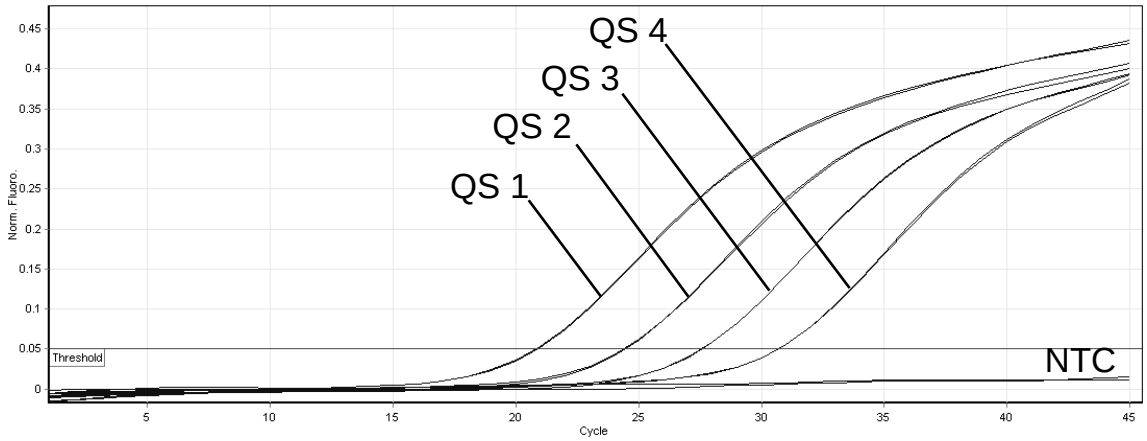

Figure 12. Detection of the quantitation standards (BK Virus RG QS 1–4) in fluorescence channel Cycling Green. NTC: No template control (negative control).

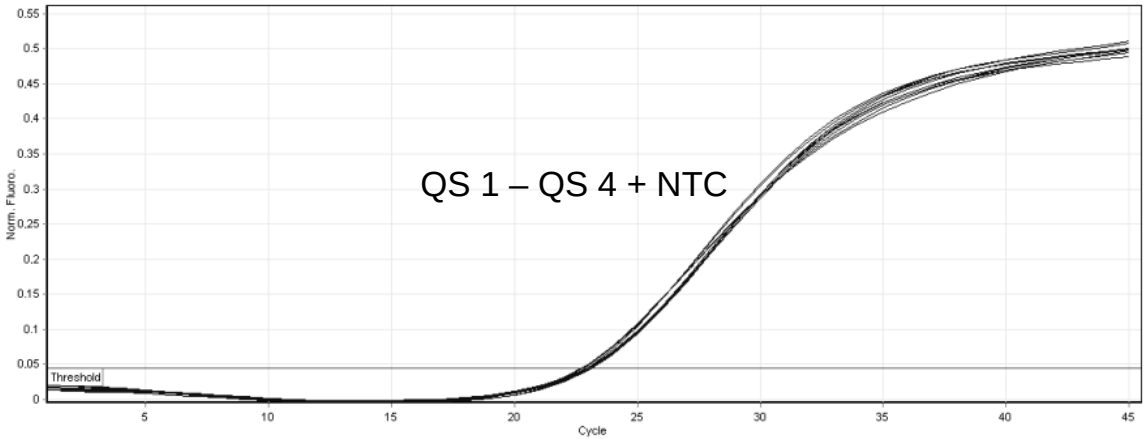

Figure 13. Detection of the internal control (IC) in fluorescence channel Cycling Orange with simultaneous amplification of the quantitation standards (BK Virus RG QS 1–4). NTC: No template control (negative control).

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
General handling	
Error message displayed in the touchscreen	If an error message is displayed during a protocol run, refer to the user manuals supplied with your instruments.
Precipitate in reagent trough of opened cartridge of the QIAsymphony DSP Virus/Pathogen Midi Kit	
a) Buffer evaporation	Excessive evaporation may lead to increased salt concentration or decreased alcohol concentrations in buffers. Discard reagent cartridge (RC). Make sure to seal buffer troughs of a partially used reagent cartridge (RC) with Reuse Seal Strips when not being used for purification.
b) Storage of reagent cartridge (RC)	Storage of reagent cartridge (RC) under 15°C may lead to formation of precipitates. If necessary, remove the troughs containing Buffers QSL2 and QSB1 from the reagent cartridge (RC) and incubate in a water bath* at 37°C for 30 min with occasional shaking to dissolve precipitate. Make sure to replace the troughs in the correct positions. If the reagent cartridge (RC) is already pierced, make sure that the troughs are reclosed with Reuse Seal Strips and incubate the complete reagent cartridge (RC) in a water bath* at 37°C for 30 min with occasional shaking.

* Ensure that instruments have been checked, maintained, and calibrated regularly according to the manufacturer's instructions.

	Comments and suggestions
Low yield of nucleic acids	
a) Magnetic particles were not completely resuspended	Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex for at least 3 min before use.
b) Frozen samples were not mixed properly after thawing	Thaw frozen samples with mild agitation to ensure thorough mixing.
c) Carrier RNA (CARRIER) not added	Reconstitute carrier RNA (CARRIER) in Buffer AVE (AVE) and mix with appropriate volume of Buffer AVE (AVE) as described in "Preparing carrier RNA (CARRIER)–Buffer AVE (AVE) mixtures" starting on page 17. Repeat the purification procedure with new samples.
d) Degraded nucleic acids	Samples were stored incorrectly or subjected to too many freeze–thaw cycles. Repeat the purification procedure with new samples.
e) Incomplete sample lysis	Before use, check that Buffer QSL2 and QSB1 do not contain precipitates. If necessary, remove the troughs containing Buffers QSL1 and QSB1 from the reagent cartridge (RC) and incubate for 30 min at 37°C with occasional shaking to dissolve precipitate. If the reagent cartridge (RC) is already pierced, make sure that the troughs are reclosed with Reuse Seal Strips, and incubate the complete reagent cartridge (RC) for 30 min at 37°C with occasional shaking in a water bath.*
f) Clogging of pipet tip due to insoluble material	Insoluble material was not removed from the sample prior to starting the QIAsymphony purification procedure. To remove insoluble material for viral applications, centrifuge the sample at 3000 x *g* for 1 min, and transfer the supernatant to a new sample tube.

* Ensure that instruments have been checked, maintained, and calibrated regularly according to the manufacturer's instructions.

	Comments and suggestions
QIAsymphony AS detects insufficient Master	
Not all of the BK Virus RG Master transferred to tube	Combine all tubes of BK Virus RG Master in a single kit into one tube. Viscous reagents can be difficult to handle with manual pipets. Make sure to transfer the entire volume of BK Virus RG Master in the tube. For viscous reagents, we recommend aspirating an extra volume of 5% when using manual pipets (e.g., adjust the pipet to 840 µl for an 800 µl volume). Alternatively, after slowly dispensing the liquid and performing a blowout at the target tube's wall, remove the tip from the liquid, release the pipet plunger, and wait for an additional 10 s. Residual liquid will flow down the tip and can be blown out by pressing the pipet plunger a second time. The use of PCR grade filter-tips labeled as "low retention" can improve the recovery of liquid.
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 on the Rotor-Gene Q", page 33.
c) Incorrect configuration of the PCR	Make sure that assay setup was performed correctly and that the correct Assay Parameter Set was used. Repeat the PCR, if necessary. See "Protocol: Assay Setup on the QIAsymphony AS", page 28.

	Comments and suggestions
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 QS-RGQ 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 sample subjected to purification using the QIAsymphony DSP Virus/Pathogen Midi Kit 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.
b) The PCR was inhibited	Make sure that you use the recommended isolation method (see "Protocol: DNA Isolation on the QIAsymphony SP", page 21) and closely follow the instructions.
c) DNA was lost during 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 "Protocol: DNA Isolation on the QIAsymphony SP", page 21) and closely follow the instructions. See also "Low yield of nucleic acids", above.
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 QS-RGQ Kit has expired	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.

Comments and suggestions	
Signals with the negative controls in fluorescence channel Cycling Green 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 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 QS-RGQ Kit (24)	For 24 reactions: Master, Magnesium Solution, 4 Quantitation Standards, Internal Control, Water (PCR grade)	4514303
QIAsymphony DSP Virus/Pathogen Midi Kit — for automated purification of viral nucleic acids or pathogen DNA from 1–96 samples		
QIAsymphony DSP Virus/Pathogen Midi Kit (96)	For up to 96 preps of 1000 µl each: Includes 2 reagent cartridges and enzyme racks and accessories	937055
QIAsymphony RGQ system		
QIAsymphony RGQ, System	QIAsymphony SP, QIAsymphony AS, Rotor-Gene Q MDx 5plex HRM, required accessories and consumables, installation and training	9001850
QIAsymphony SP/AS instruments and accessories		
QIAsymphony SP	QIAsymphony sample prep module, 1-year warranty on parts and labor	9001297
QIAsymphony AS	QIAsymphony assay setup module, 1-year warranty on parts and labor	9001301
Buffer ATL (4 x 50 ml)	4 x 50 ml Tissue Lysis Buffer for use with QIAsymphony SP pathogen complex protocols	939011
Sample Prep Cartridges, 8-well (336)	8-well sample prep cartridges for use with the QIAsymphony SP	997002
8-Rod Covers (144)	8-Rod Covers for use with the QIAsymphony SP	997004
Cooling Adapter, EMT, v2, Qsym	Cooling adapter for EMT racks; for use with the QIAsymphony SP/AS instruments (software version 3.1 or higher)	920730
Reuse Seal Set (20)	Reuse seal sets for sealing partly used QIAsymphony reagent cartridges	997006

Product	Contents	Cat. no.
Insert, 2.0ml v2, samplecarr. (24), Qsym	Secondary tube adapter (for 2 ml screw-cap tubes, tube insert 3b) for use with the QIAsymphony SP tube carrier	9242083
Elution Microtubes CL (24 x 96)	Nonsterile polypropylene tubes (0.85 ml maximum capacity, less than 0.7 ml storage capacity, 0.4 ml elution capacity); 2304 in racks of 96; includes cap strips	19588
Cooling Adapter, Reagent Holder 1, Qsym	Adapter for holding 18 x 2 ml conical tubes, and 6 x 5 ml conical tubes; for use with the QIAsymphony SP/AS instruments (software version 3.1 or higher)	9018090
Cooling Adapter, Reagent Holder 2, Qsym	Adapter for holding 18 x 2 ml conical tubes, 2 x 5 ml conical tubes, and 2 x Reagent Bottles, 30 ml; for use with the QIAsymphony SP/AS instruments (software version 3.1 or higher)	9018089
Cooling Adapter, RG Strip Tubes 72, Qsym	Adapter for holding 18 strips of 4 tubes; for use with the QIAsymphony SP/AS instruments (software version 3.1 or higher)	9018092
Tubes, conical, 2 ml, Qsym AS (500)	Conical tubes (2 ml) for holding reagent; for use with the QIAsymphony AS	997102*
Tube, conical, 5 ml, Qsym AS (500)	Conical tubes (5 ml) for holding reagent; for use with the QIAsymphony AS	997104*
Reagent Bottles, 30 ml, Qsym AS (50)	Reagent bottles (30 ml) with lids; for use with the QIAsymphony AS	997108
Filter-Tips, 1500 µl (1024)	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAsymphony SP	997024
Filter-Tips, 200 µl (1024)	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAcube and the QIAsymphony SP	990332

* Please inquire for availability.

Product	Contents	Cat. no.
Filter-Tips, 50 µl (1024)	Disposable Filter-Tips, racked (8 x 128). For use with the QIAsymphony AS	997120
Tip Disposal Bags (15)	Tip disposal bags for use with the QIAsymphony SP/AS instruments	9013395
Rotor-Gene Q and accessories		
Rotor-Gene Q MDx 5plex HRM System	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor	Inquire
Rotor-Gene Q MDx 5plex HRM Platform	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor; includes installation and training	Inquire
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	9001650
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; includes installation and training	9001580
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

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