May 2010

artus® HSV-1/2 RG PCR Kit Handbook

24 (catalog no. 4500203)

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

For use with Rotor-Gene® Q instruments

REF 4500203

HB 1060170

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Contents

Kit Contents

***artus* HSV-1/2 RG PCR Kit**			**(24)**
Catalog no.			**4500203**
Number of reactions			**24**
Blue	HSV-1/2 RG Master		2 x 300µl
Yellow	HSV-1/2 RG Mg-Sol*	Mg-Sol	600 µl
Red	HSV-1 RG PC† (100 cop/µl)		200 µl
Brown	HSV-2 RG PC† (100 cop/µl)		200 µl
Green	HSV-1/2 RG IC‡	IC	1000 µl
White	Water (PCR grade)		1000 µl
	Handbook	HB	1

* Magnesium solution.

† Positive control

‡ Internal control.

Symbols

Σ <N>	Contains reagents for <N> tests
	Use by
REF	Catalog number
LOT	Lot number
MAT	Material number
COMP	Components
CONT	Contains
NUM	Number

Temperature limitations

Legal manufacturer

Refer to information given in the handbook

Storage

The components of the *artus* HSV-1/2 RG PCR Kit should be stored at –20°C and are stable until the expiration date stated on the label. Repeated thawing and freezing (>2 x) should be avoided, as this may reduce assay sensitivity. If the reagents are to be used only intermittently, they should be frozen in aliquots. Storage at 2–8°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* HSV-1/2 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.

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At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the *artus* HSV-1/2 RG PCR Kit or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Safety Information

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

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

24-hour emergency information

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

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Introduction

The *artus* HSV-1/2 RG PCR Kit constitutes a ready-to-use system for the detection of HSV-1 and HSV-2 DNA using polymerase chain reaction (PCR) on Rotor-Gene Q instruments. The HSV-1/2 RG Master contains reagents and enzymes for the specific amplification of a 154 bp region of the HSV-1 and HSV-2 genome, and for the direct detection of the specific amplicon in fluorescence channel Cycling Green (source 470 nm, detector 510 nm) and Cycling Orange (source 585 nm, detector 610 nm) of the Rotor-Gene Q instruments.

In addition, the *artus* HSV-1/2 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 (source 530 nm, detector 555 nm) of the Rotor-Gene Q instruments. The detection limit of the analytical HSV-1/2 RG PCR is not reduced. External positive controls (HSV-1 RG PC and HSV-2 RG PC) are supplied.

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

Herpes simplex virus (HSV) is found in lesion fluids, saliva, cerebrospinal fluid (CSF), and vaginal secretions. It is transmitted primarily by direct contact with lesions and via sexual intercourse, as well as perinatally. Lesions on the skin and mucous membranes of the mouth and genitals characterize most HSV positive cases. HSV infection can be either primary (> 90 % of these cases are asymptomatic) or recurrent (secondary). Primary infection with HSV-1 can lead to, among others, gingivostomatitis, eczema herpeticum, keratoconjunctivitis and encephalitis; primary HSV-2 infection occurs as, among others, vulvovaginitis, meningitis and generalized herpes in newborns. The primary symptoms of a secondary infection are skin lesions in the nose, mouth and genital regions. Even more severe are the recurrent forms of keratoconjunctivitis and meningitis.

* 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, Cycling Orange and Cycling Yellow
- Rotor-Gene Q software version 1.7.94, and higher (Rotor-Gene 6000 software version 1.7.65, and higher)
- 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* HSV-1/2 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) is validated for viral DNA purification from human CSF for use with the *artus* HSV-1/2 RG PCR Kit. Carry out the viral DNA purification according to the instructions in the *EZ1 DSP Virus Kit Handbook*.

Note: The *artus* HSV-1/2 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 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* HSV-1/2 RG PCR Kit can be used directly in the isolation procedure (see "Internal control", page 11).

Internal control

An internal control (HSV-1/2 RG IC) is supplied. This allows the user both to control the DNA isolation procedure and to check for possible PCR inhibition. For this application, add the internal control to the isolation at a ratio of 0.1 µl per 1 µl elution volume. For example, using the EZ1 DSP Virus Kit, the DNA is eluted in 60 µl Elution Buffer (AVE). Hence, 6 µl of the internal control should be added initially.

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 HSV-1/2 RG Master and HSV-1/2 RG Mg-Sol, as described in step 2b of the protocol (page 13).

Protocol: PCR and Data Analysis

Important points before starting

- Before beginning the procedure, read "Important Notes", page 10.
- Take time to familiarize yourself with the Rotor-Gene Q instrument before starting the protocol. See the instrument user manual.
- Make sure that the positive controls and one negative control (Water, PCR grade) are included per 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.
   Use the internal control according to step 2b for all positive and negative controls.

2a. The internal control has already been added to the isolation (see "Internal control", page 11). 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. PCR set up for PC and NTC should contain IC. Therefore, a second master mix containing IC according to Table 2 below is required for NTC and PC.

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

Number of samples	1	12
HSV-1/2 RG Master	25 µl	300 µl
HSV-1/2 RG Mg-Sol	5 µl	60 µl
HSV-1/2 RG IC	0 µl	0 µl
Total volume	**30 µl**	**360 µl**

2b. The internal control must be added directly to the mixture of HSV-1/2 RG Master and HSV-1/2 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
HSV-1/2 RG Master	25 µl	300 µl
HSV-1/2 RG Mg-Sol	5 µl	60 µl
HSV-1/2 RG IC	2 µl	24 µl
Total volume	**32 µl***	**384 µl***

* The volume increase caused by adding the internal control is neglected when preparing the PCR assay. The sensitivity of the detection system is not impaired.

3. Pipet 30 µl of the master mix into each PCR tube. Then add 20 µl of the eluted sample DNA (see Table 3 below), and mix well by pipetting repeatedly up and down. Correspondingly, 20 µl of the HSV-1 RG PC and HSV-2 RG PC have to be used as positive controls and 20 µl of water (Water, PCR grade) as a negative control.

Table 3. Preparation of PCR assay

Number of samples	1	12
Master mix	30 µl	30 µl each
Sample	20 µl	20 µl each
Total volume	**50 µl**	**50 µl each**

4. **Close the PCR tubes. Make sure that the locking ring (accessory of the Rotor-Gene instrument) is placed on top of the rotor to prevent accidental opening of the tubes during the run.**
5. **For the detection of HSV-1 DNA or HSV-2 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 higher, Rotor-Gene 6000 software versions 1.7.65, and higher. 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) with the "Advanced" version. Check the "Locking Ring Attached" box and click "Next".**

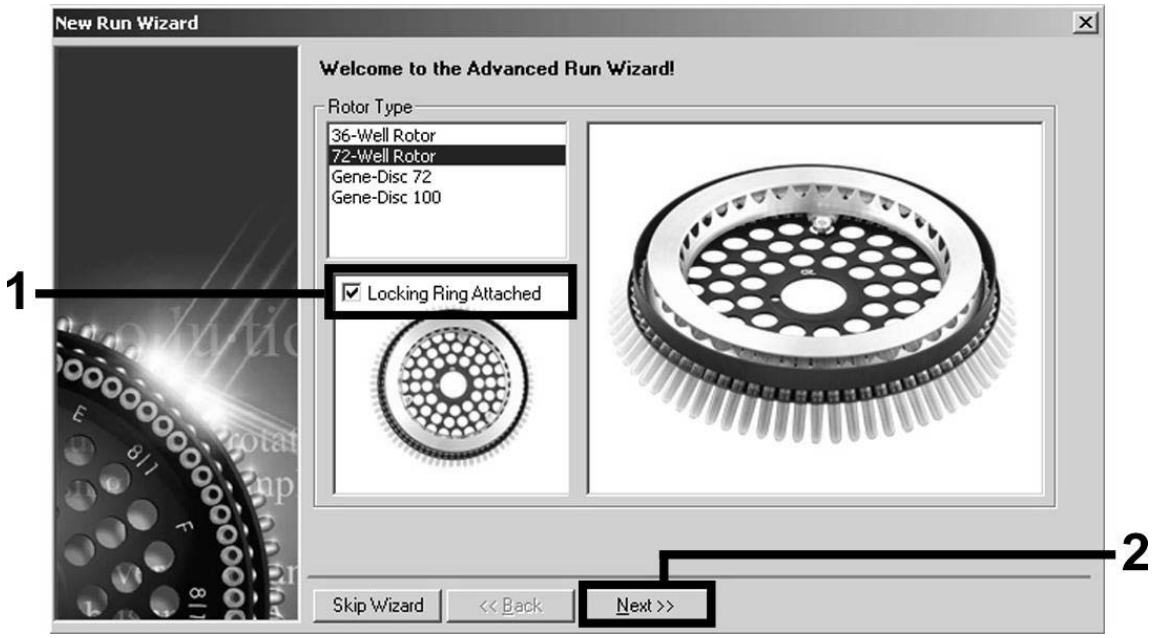

Figure 1. The "New Run Wizard" dialog box.

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

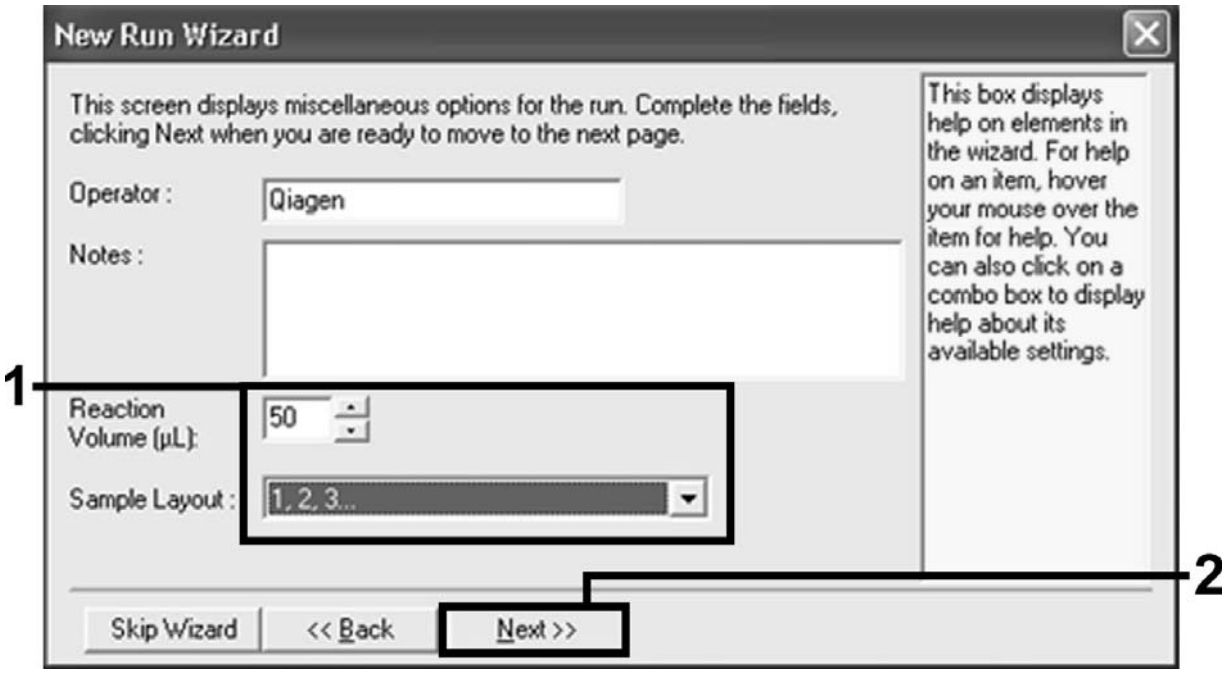

Figure 2. Setting the general assay parameters.

8. **Click the "Edit Profile" button in the next "New Run Wizard" dialog box (Figure 3), and program the temperature profile as shown in Figures 4–5.**

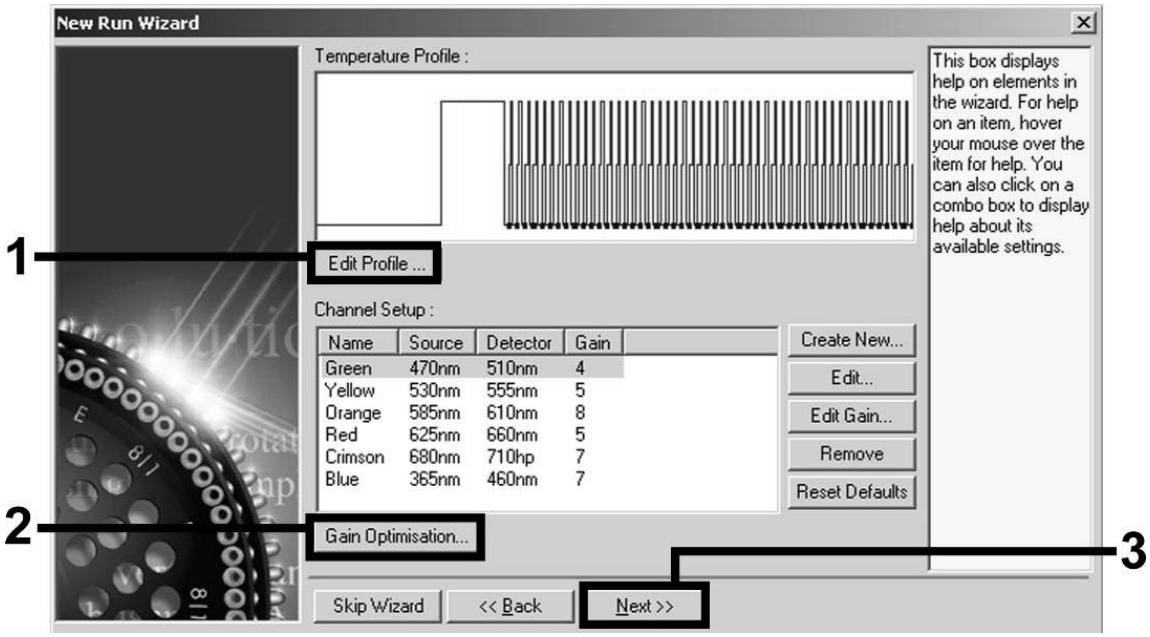

Figure 3. Editing the profile.

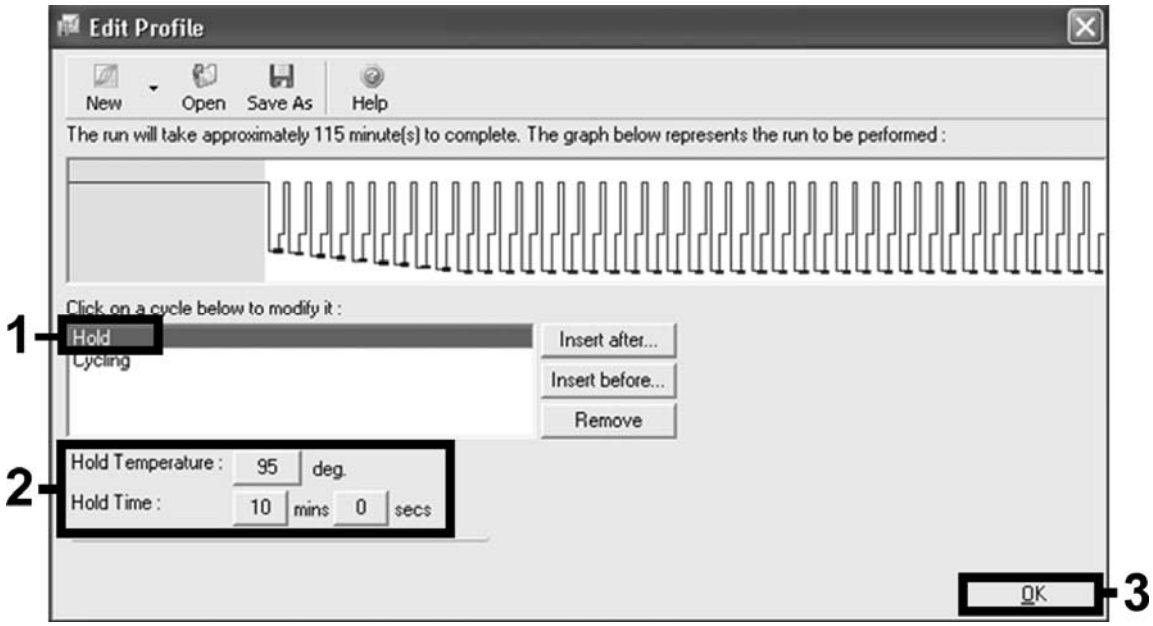

Figure 4. Initial activation of the hot-start enzyme.

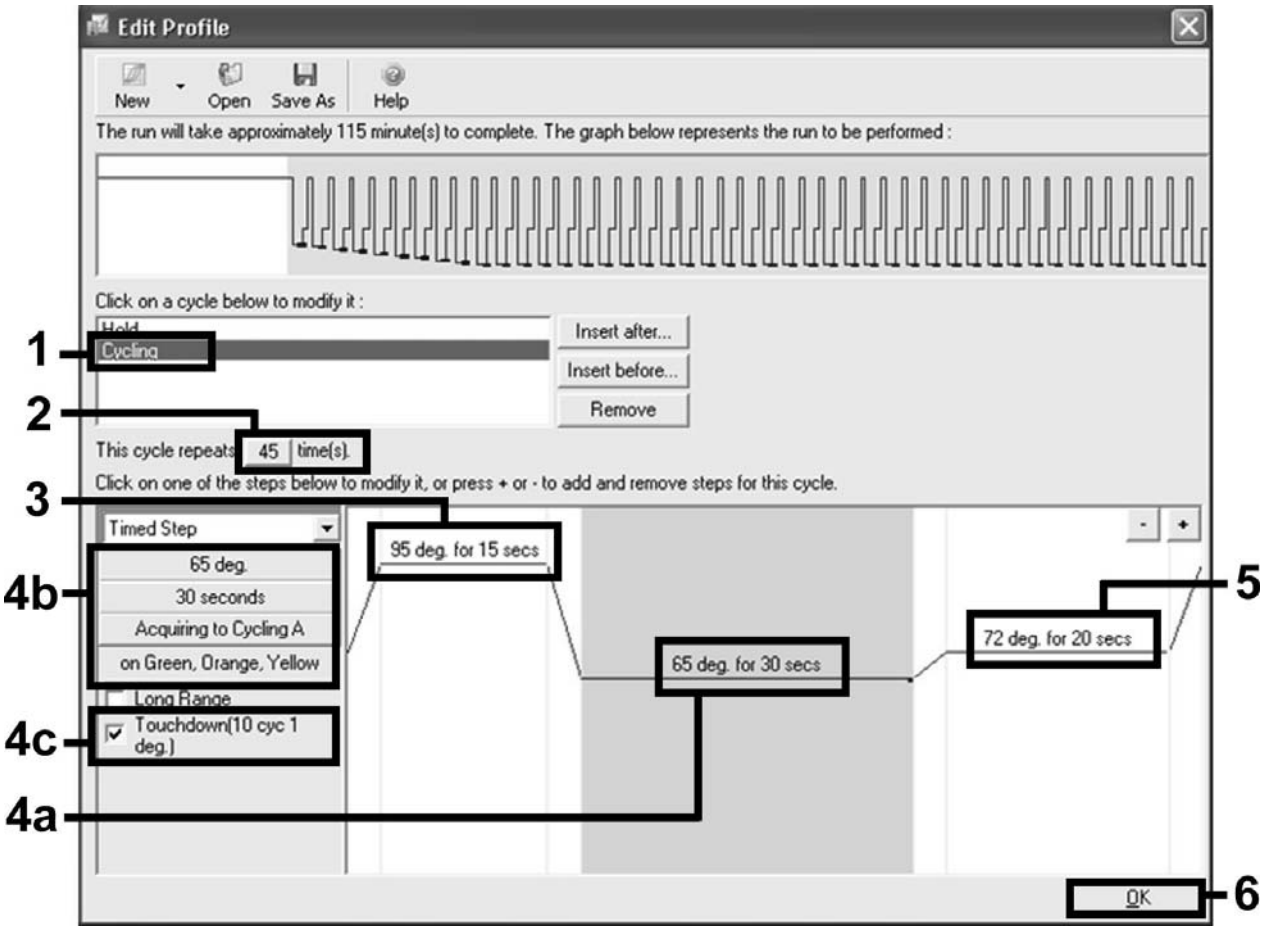

Figure 5. Amplification of the DNA. Make sure to activate the touchdown function for 10 cycles in the Annealing step.

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, Step 2) to open the "Auto-Gain Optimisation Setup" dialog box (Figure 6). Set the calibration temperature to *65* to match the annealing temperature of the amplification program (Figure 5, Step 4b). Make sure that all three channels (Green, Orange, and Yellow) are selected for "Auto-Gain Optimisation". (Find channels in the drop down menu under "Channel Settings" and click "Add".) Click "Start" to begin the gain optimization. Click "Close" of the "Auto-Gain Optimisation Setup" dialog box when the gain calibration is completed.

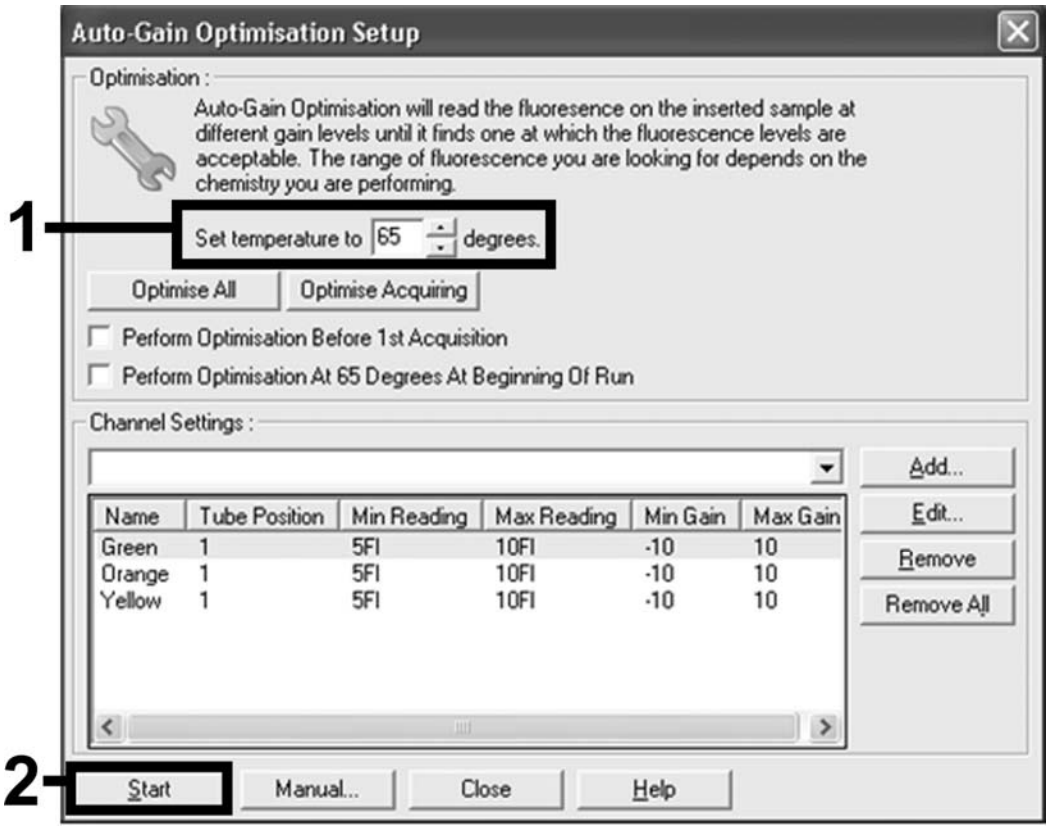

Figure 6. Adjusting the fluorescence channel sensitivity.

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".

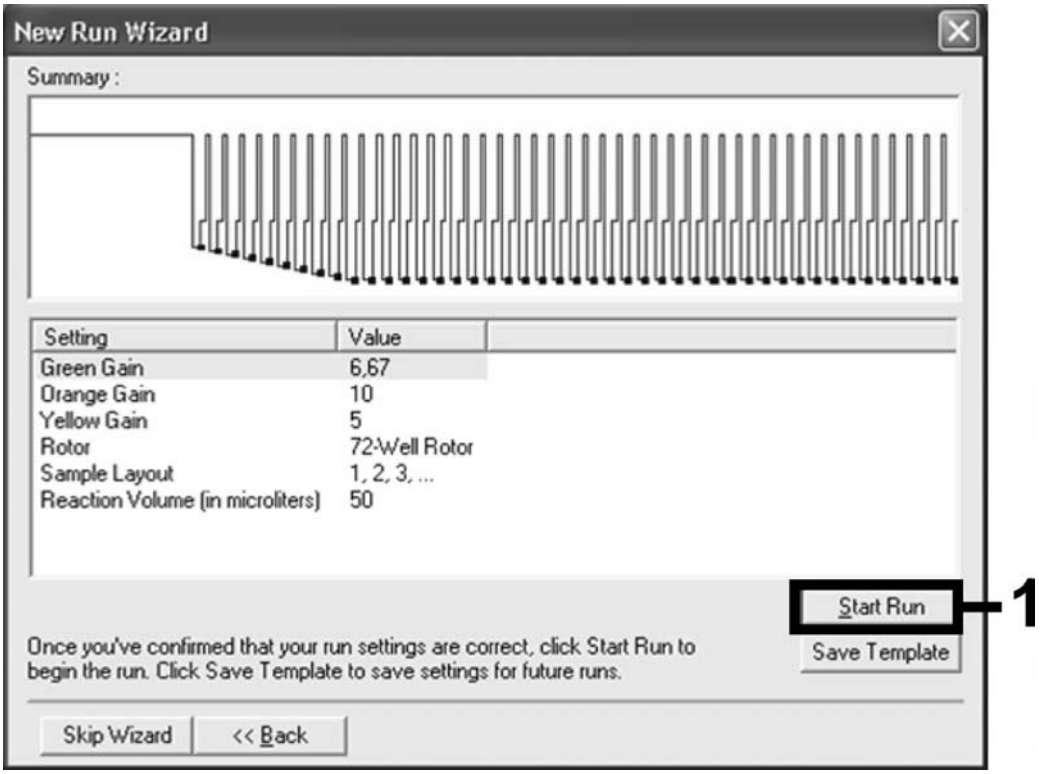

Figure 7. Starting the run.

11. After the run is finished, analyze the data. The following results (11a, 11b, 11c, 11d, 11e, and 11f) are possible.

Examples of positive and negative PCR reactions are given in Figure 8, 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 HSV-1 DNA.

In this case, the detection of a signal in the Cycling Yellow channel is dispensable, since high initial concentrations of HSV-1 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).

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 HSV-1 DNA is detectable. It can be considered HSV-1 negative.

In the case of a negative HSV-1 PCR, the detected signal of the internal control rules out the possibility of PCR inhibition.

11c. A signal is detected in fluorescence channel Cycling Orange. The result of the analysis is positive: the sample contains HSV-2 DNA.

In this case, the detection of a signal in the Cycling Yellow channel is dispensable, since high initial concentrations of HSV-2 DNA (positive signal in the Cycling Orange channel) can lead to a reduced or absent fluorescence signal of the internal control in the Cycling Yellow channel (competition).

11d. In fluorescence channel Cycling Orange no signal is detected. At the same time, a signal from the internal control appears in the Cycling Yellow channel. In the sample no HSV-2 DNA is detectable. It can be considered HSV-2 negative.

In the case of a negative HSV-2 PCR, the detected signal of the internal control rules out the possibility of PCR inhibition.

11e. A signal is detected in the Cycling Green and Cycling Orange channels. The result of the analysis is positive: the sample contains HSV-1 DNA as well as HSV-2 DNA.

In this case, the detection of a signal in the Cycling Yellow channel is dispensable, since high initial concentrations of both HSV-1 and HSV-2 DNA (positive signal in the Cycling Green and Cycling Orange channel)

can lead to a reduced or absent fluorescence signal of the internal control in the Cycling Yellow channel (competition).

11f. No signal is detected in the Cycling Green, Cycling Orange 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 22.

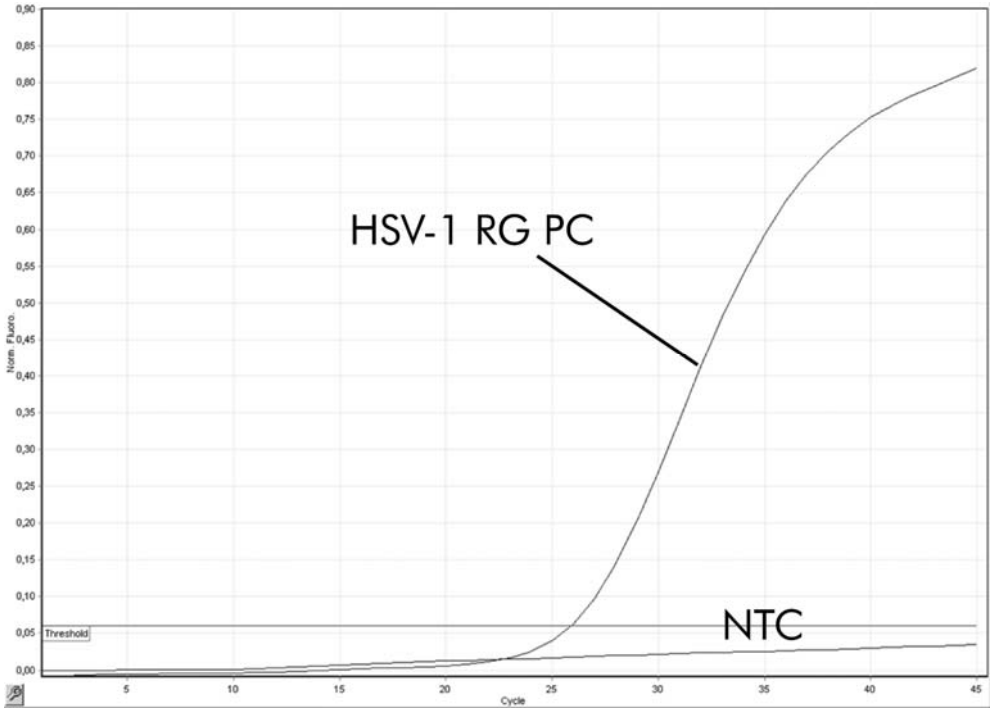

Figure 8. Detection of the HSV-1 positive control (HSV-1 RG PC) in fluorescence channel Cycling Green. NTC: No template control (negative control).

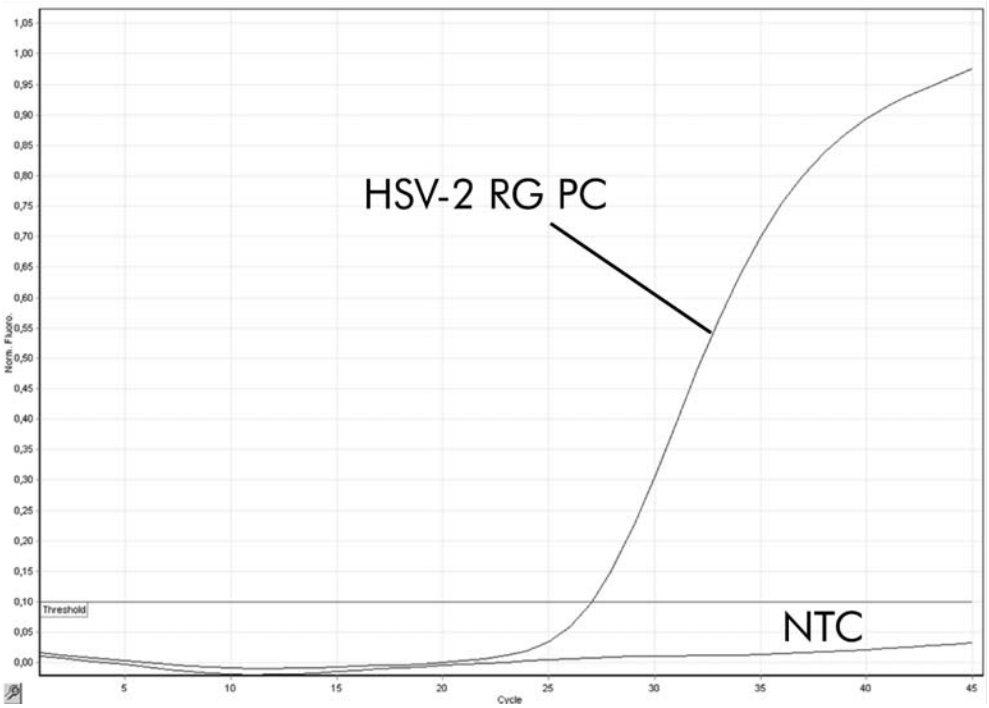

Figure 9. Detection of the HSV-2 positive control (HSV-2 RG PC) in fluorescence channel Cycling Orange. NTC: No template control (negative control).

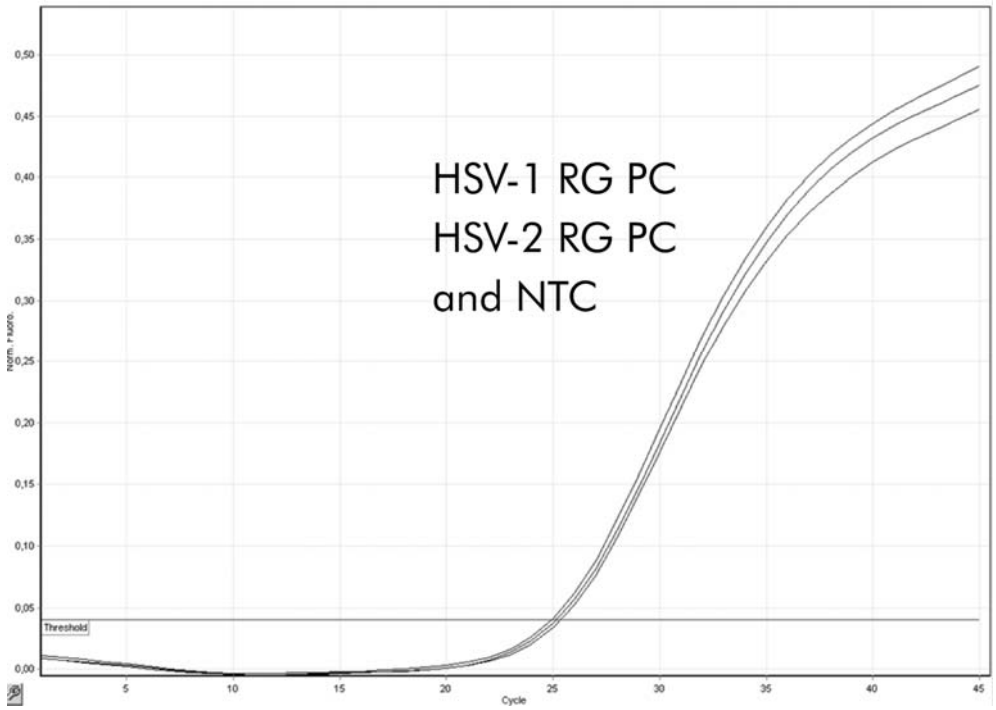

Figure 10. Detection of the internal control (IC) in fluorescence channel Cycling Yellow with simultaneous amplification of the positive controls (HSV-1 RG PC and HSV-2 RG PC). NTC: No template control (negative control).

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 (HSV-1 RG PC and HSV-2 RG PC) in fluorescence channel Cycling Green or Cycling Orange	
a) The selected fluorescence channel for PCR data analysis does not comply with the protocol	For data analysis select the fluorescence channel Cycling Green and Cycling Orange for the analytical HSV-1/2 PCR and the fluorescence channel Cycling Yellow 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* HSV-1/2 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 CSF sample subjected to purification using the EZ1 DSP Virus Kit in fluorescence channel Cycling Yellow and simultaneous absence of a signal in channel Cycling Green or Cycling Orange	
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* HSV-1/2 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 Orange 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 HSV-1/2 RG PCR Kit (24)	For 24 reactions: Master, Mg Solution, 2 Positve Controls, Internal Control, Water (PCR grade)	4500203
EZ1 DSP Virus Kit — for purification of viral nucleic acids from human CSF for in vitro diagnostic purposes		
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
Rotor-Gene Q and accessories		
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
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

Product	Contents	Cat. no.
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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Trademarks: QIAGEN®, *artus*®, EZ1® (QIAGEN Group); Rotor-Gene® (Corbett Research Pty Ltd).

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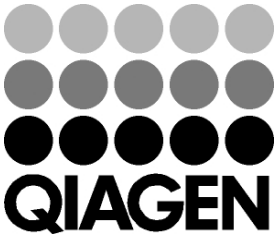

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