March 2010

artus® Infl./H1 LC/RG RT-PCR Kit Handbook

Σ 24 (catalog no. 4523003)

Σ 96 (catalog no. 4523005)

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

For use with Rotor-Gene® Q instruments or LightCycler® 1.1/1.2/1.5/2.0 instruments

REF 4523003, 4523005

H B 1062779

QIAGEN GmbH, QIAGEN Strasse 1, D-40724 Hilden

MAT 1062779

QIAGEN®

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

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

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com.

Contents

Kit Contents

***artus* Infl./H1 LC/RG RT-PCR Kit**			**(24)**	**(96)**
Catalog no.			**4523003**	**4523005**
Number of reactions			**24**	**96**
Blue	Influenza Master		2 x 12 reactions	8 x 12 reactions
Violet	Influenza H1 Master		2 x 12 reactions	8 x 12 reactions
Yellow	Influenza Mg-Sol*	**Mg-Sol**	800 µl	800 µl
Red	Influenza Control		200 µl	200 µl
Brown	Influenza H1 Control		200 µl	200 µl
Green	Influenza IC†	**IC**	1000 µl	2 x 1000 µl
White	Water (PCR grade)		1000 µl	1000 µl
	Handbook	HB	1	1

* Magnesium solution.

† Internal control.

Symbols

<N>

Contains reagents for <N> tests

Use by

Catalog number

Lot number

Material number

Components

Contains

Number

Temperature limitations

Legal manufacturer

Refer to information given in the handbook

Storage

The components of the *artus* Infl./H1 LC/RG RT-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.

Technical Assistance

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* Infl./H1 LC/RG RT-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).

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.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of *artus* Infl./H1 LC/RG RT-PCR Kit is tested against predetermined specifications to ensure consistent product quality.

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* Infl./H1 LC/RG RT-PCR Kit constitutes two ready-to-use systems for the detection of influenza A and B viral RNA and novel influenza A (H1N1) viral RNA (2009 H1N1 virus) using reverse transcription–polymerase chain reaction (RT-PCR) on Rotor-Gene Q or LightCycler instruments. The Influenza Master contains reagents and enzymes for the specific amplification of a 143 bp region of the influenza virus A genome and a 94 bp region of the influenza virus B genome, and for the direct detection of the specific amplicon in fluorescence channel Cycling Green of the Rotor-Gene Q or Rotor-Gene 6000, fluorescence channel Cycling A.FAM® (source 470 nm, detector 510 nm) of the Rotor-Gene 3000, fluorescence channel 530 of the LightCycler 2.0 instrument, or channel F1 of the LightCycler 1.5 instrument.

Note: The *artus* Infl./H1 LC/RG RT-PCR Kit may not be used with Rotor-Gene Q 2plex Instruments.

In addition, the *artus* Infl./H1 LC/RG RT-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, fluorescence channel Cycling A.ROX™ (source 585 nm, detector 610 nm) of the Rotor-Gene 3000, fluorescence channel 610 of the LightCycler 2.0 instrument, or channel F2 of the LightCycler 1.5 instrument.

The Influenza H1 Master contains reagents and enzymes for the specific amplification of a 80 bp region of influenza virus H1 (2009 H1N1 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, fluorescence channel Cycling A.FAM (source 470 nm, detector 510 nm) of the Rotor-Gene 3000, fluorescence channel 530 of the LightCycler 2.0 instrument, or channel F1 of the LightCycler 1.5 instrument. Two external positive controls are supplied (Influenza Control and Influenza H1 Control).

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 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.*

Background information

Influenza viruses are enveloped single-stranded RNA viruses that belong to the Orthomyxoviridae family. The major surface antigens are hemagglutinin (H) and neuraminidase (N). The surface antigens change continuously (antigenic drift).

The *artus* Infl./H1 LC/RG RT-PCR Kit contains an Influenza Master for detection of influenza A and B viruses. The kit also contains an influenza A (H1N1) specific detection reagent, enabling additional detection of the 2009 H1N1 virus. This detection reagent contains primers and probes for the detection of hemagglutinin 1 (H1) sequences that are specific to the 2009 H1N1 virus.

* 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.

- RNA isolation kit (see "RNA isolation", page 10)
- Pipets (adjustable)*
- Sterile pipet tips with filters
- Vortex mixer*
- Benchtop centrifuge* with rotor for 2 ml reaction tubes

Using Rotor-Gene instruments

- Rotor-Gene Q or Rotor-Gene instrument*† with fluorescence channels for Cycling Green and Cycling Orange or with fluorescence channels for Cycling A.FAM and Cycling A.ROX
- Rotor-Gene Q software version 1.7.94 or higher (Rotor-Gene 6000 software version 1.7.65, 1.7.87, 1.7.94; Rotor-Gene 3000 software version 6.0.23)
- Strip Tubes and Caps, 0.1 ml, for use with 72-well rotor (cat. no. 981103 or 981106)
- Alternatively: PCR Tubes, 0.2 ml, for use with 36-well rotor (cat. no. 981005 or 981008)
- Cooling block (Loading Block 72 x 0.1 ml Tubes, cat. no. 9018901, or Loading Block 96 x 0.2 ml Tubes, cat. no. 9018905)

Using LightCycler instruments

- LightCycler 1.1/1.2/1.5 (Software Version 3.5) or LightCycler 2.0 (Software Version 4.0) Instrument,* Roche Diagnostics
- Color Compensation Set (Roche Diagnostics, cat. no. 2 158 850) for the installation of a Crosstalk Color Compensation file
- LightCycler Multicolor Demo Set (Roche Diagnostics, cat. no. 03 624 854 001) for the LightCycler 2.0 Instrument
- LightCycler Capillaries (20 µl), Roche Diagnostics
- LightCycler Cooling Block, Roche Diagnostics
- LightCycler Capping Tool, Roche Diagnostics

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

† The *artus* Infl./H1 LC/RG RT-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).

RNA isolation

The kits from QIAGEN shown in Table 1 are recommended for viral RNA purification from the indicated human sample types for use with the *artus* Infl./H1 LC/RG RT-PCR Kit. Carry out the viral RNA purification according to the instructions in the kit handbooks.

Table 1. Purification kits recommended for use with the *artus* Infl./H1 LC/RG RT-PCR Kit

Sample material	Nucleic acid isolation kit	Catalog number (QIAGEN)	Carrier RNA
Sputum; throat and nasal swabs in viral transport medium	QIAamp® Viral RNA Mini Kit (50)	52904	Included
	QIAamp MinElute® Virus Spin Kit (50)	57704	Included
	EZ1® DSP Virus Kit (48)	62724	Included

Note: The *artus* Infl./H1 LC/RG RT-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 RNA yield. Add the appropriate amount of carrier RNA to each extraction following the instructions in the kit handbooks.

Note: The internal control of the *artus* Infl./H1 LC/RG RT-PCR Kit can be used directly in the isolation procedure (see "Internal control", page 11).

Using the QIAamp Viral RNA Mini Kit

Note: We strongly recommend to perform the recommended centrifugation step 10 in the protocol (*QIAamp Viral RNA Mini Handbook*, Third Edition, December 2007, page 25) to remove any residual ethanol. We recommend to increase the time of this centrifugation to 3 minutes.

Using the EZ1 DSP Virus Kit

The EZ1 DSP Virus Kit is to be used with one of the following combinations of EZ1 instruments and cards.

- EZ1 Advanced (cat. no. 9001411) and the EZ1 Advanced DSP Virus Card (cat. no. 9018305)
- BioRobot® EZ1 DSP Workstation (cat. no. 9001360) and the EZ1 DSP Virus Card (cat. no. 9017707)

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 (Influenza IC) is supplied. This allows the user both to control the RNA 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 QIAamp Viral RNA Mini Kit, the RNA is eluted in 60 µl Buffer AVE. Hence, 6 µl of the internal control should be added initially.

Note: The internal control and carrier RNA (see "RNA 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 Influenza Master and Influenza Mg-Sol, as described in step 2b of the protocols (pages 14 and 25).

Note: The Influenza H1 reaction mix does not contain an internal control. In some cases, the competitive IC PCR may reduce the signal of the analytical Influenza PCR. The Influenza H1 Master is therefore slightly more sensitive. We recommend testing samples with the Influenza Master and the Influenza H1 Master simultaneously in one run.

Protocol: PCR and Data Analysis (Rotor-Gene Instruments)

Important points before starting

- Before beginning the procedure, read "Important Notes", pages 10–12.
- Take time to familiarize yourself with the Rotor-Gene Q before starting the protocol. See the instrument user manual.
- Make sure that the appropriate positive control (Influenza Control or Influenza H1 Control) as well as one no template 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 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 RNA 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. For the Influenza H1 PCR, no internal control is included; follow step 2c.**

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 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 to monitor RNA isolation and check for PCR inhibition)

Number of samples	1	12
Influenza Master	12 µl	144 µl
Influenza Mg-Sol	3 µl	36 µl
Influenza IC	0 µl	0 µl
Total volume	**15 µl**	**180 µl**

2b. The internal control must be added directly to the mixture of Influenza Master and Influenza Mg-Sol. In this case, prepare a master mix according to Table 3.

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

Table 3. Preparation of master mix (internal control used exclusively to check for PCR inhibition)

Number of samples	1	12
Influenza Master	12 µl	144 µl
Influenza Mg-Sol	3 µl	36 µl
Influenza IC	0.5 µl	6 µl
Total volume	**15.5 µl***	**186 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.

2c. For the Influenza H1 PCR, no internal control is included. In this case, prepare a master mix according to Table 4.

Note: The Influenza H1 PCR should only be performed on samples that have tested positive for influenza virus RNA using the Influenza Master.

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

Table 4. Preparation of Influenza H1 master mix (no internal control)

Number of samples	1	12
Influenza H1 Master	12 µl	144 µl
Influenza Mg-Sol	3 µl	36 µl
Total volume	**15 µl**	**180 µl**

3. **Pipet 15 µl of the master mix into each PCR tube. Then add 5 µl of the eluted sample RNA (see Table 5). Correspondingly, 5 µl of the appropriate control (Influenza Control or Influenza H1 Control) must be used as a positive control and 5 µl of water (Water, PCR grade) as a no template control.**

Table 5. Preparation of PCR assay

Number of samples	1	12
Master mix	15 µl	15 µl each
Sample	5 µl	5 µl each
Total volume	**20 µl**	**20 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 influenza virus RNA, create a temperature profile according to the following steps.**

Setting the general assay parameters	**Figures 1, 2, 3**
Reverse transcription of the RNA	**Figure 4**
Initial activation of the hot-start enzyme	**Figure 5**
Amplification of the cDNA	**Figure 6**
Adjusting the fluorescence channel sensitivity	**Figure 7**
Starting the run	**Figures 8, 9**

All specifications refer to the Rotor-Gene Q software version 1.7.94 or higher, Rotor-Gene 6000 software versions 1.7.65, 1.7.87, 1.7.94, and Rotor-Gene 3000 software version 6.0.23. Please find further information on programming Rotor-Gene instruments in the instrument user manual. In the illustrations these settings are framed in bold black. Illustrations are included for Rotor-Gene Q instruments. Where different values are required for the Rotor-Gene 3000, these differences are described in the text.

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

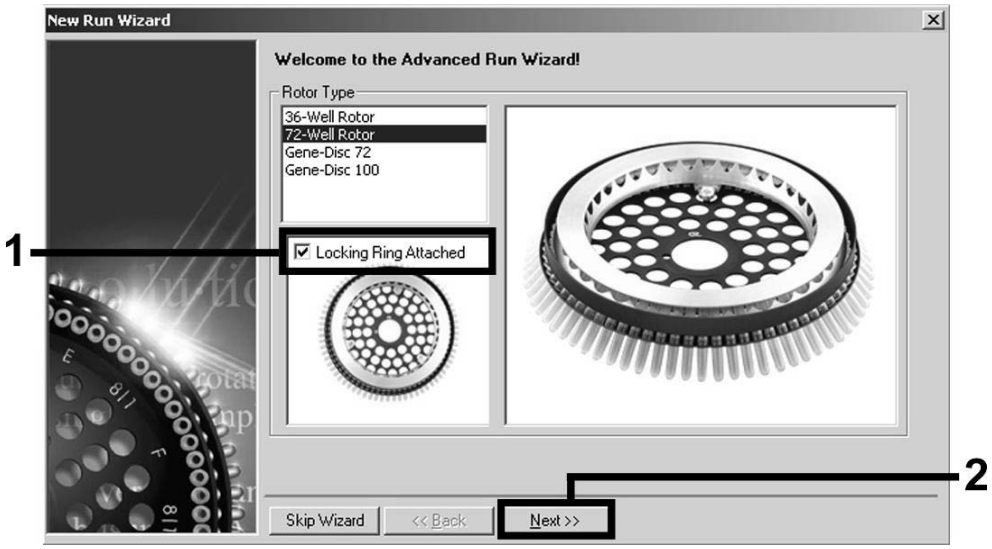

Figure 1. The "New Run Wizard" dialog box.

7. **Select *20* for the PCR reaction volume and click "Next" (Figure 2).**

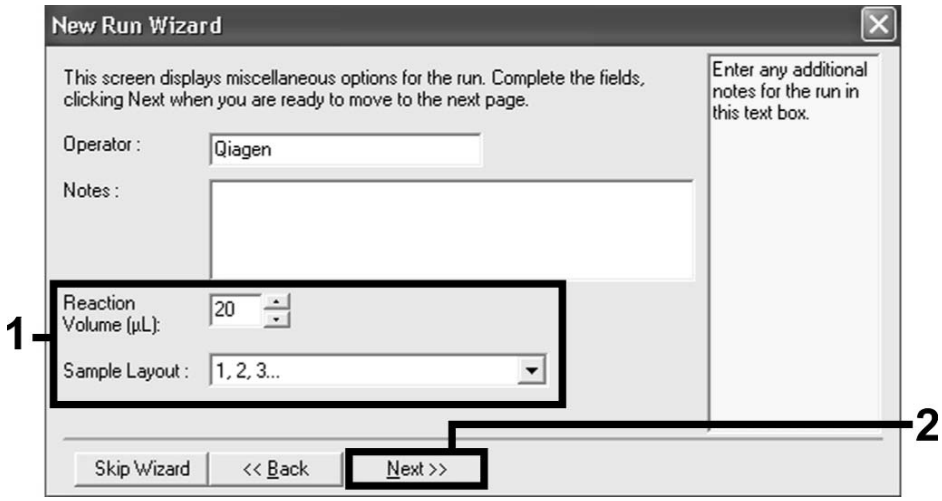

Figure 2. Setting the general assay parameters.

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 3–6).**

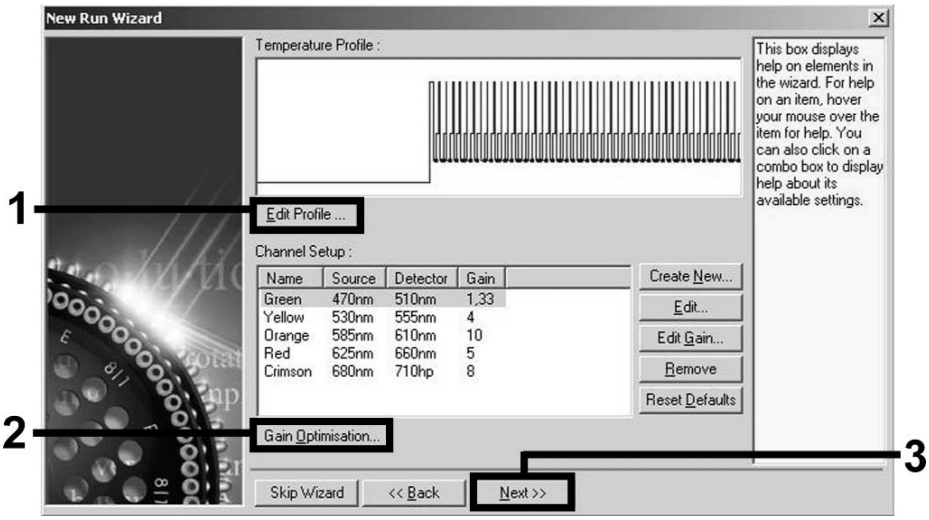

Figure 3. Editing the profile.

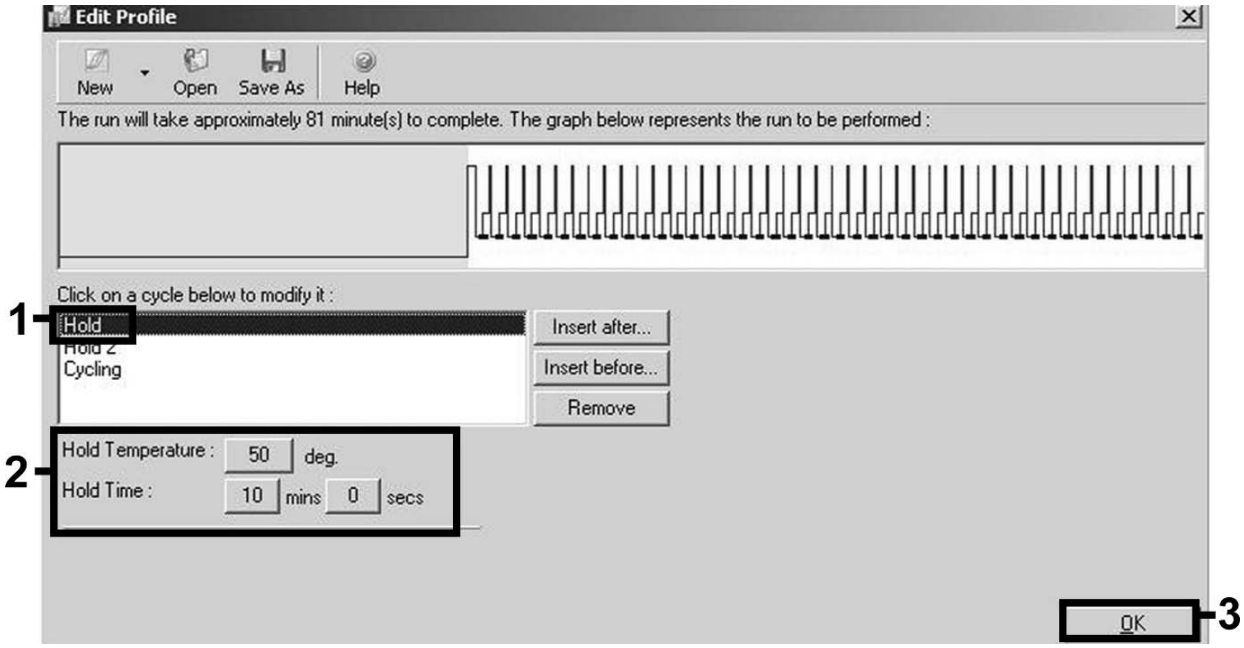

Figure 4. Reverse transcription of the RNA.

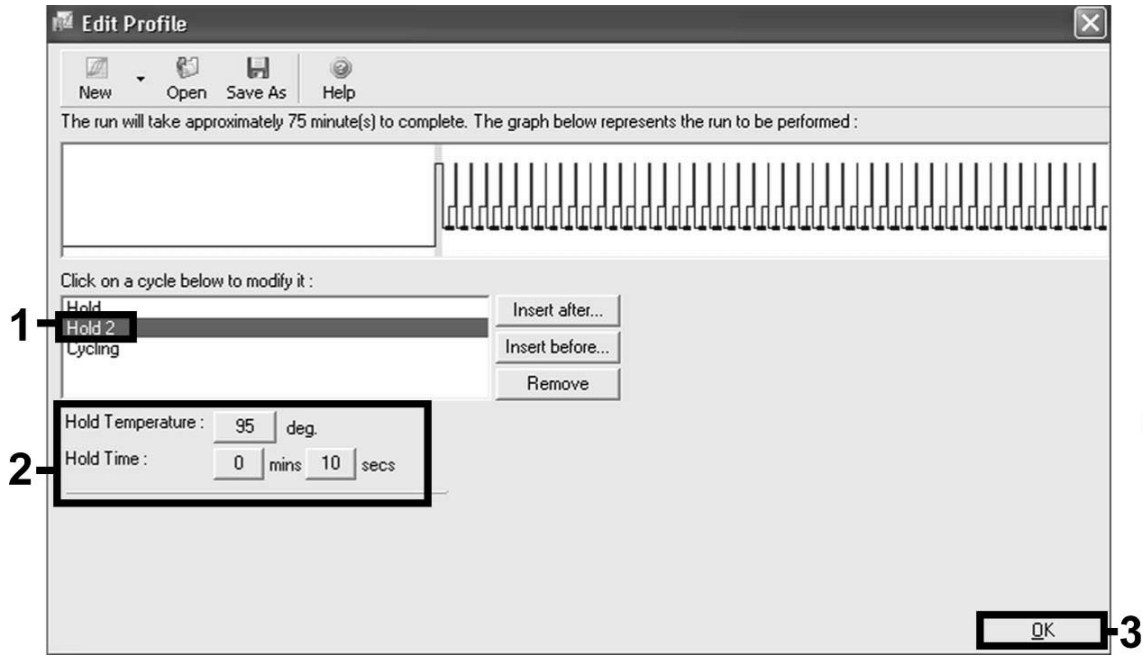

Figure 5. Initial activation of the hot-start enzyme.

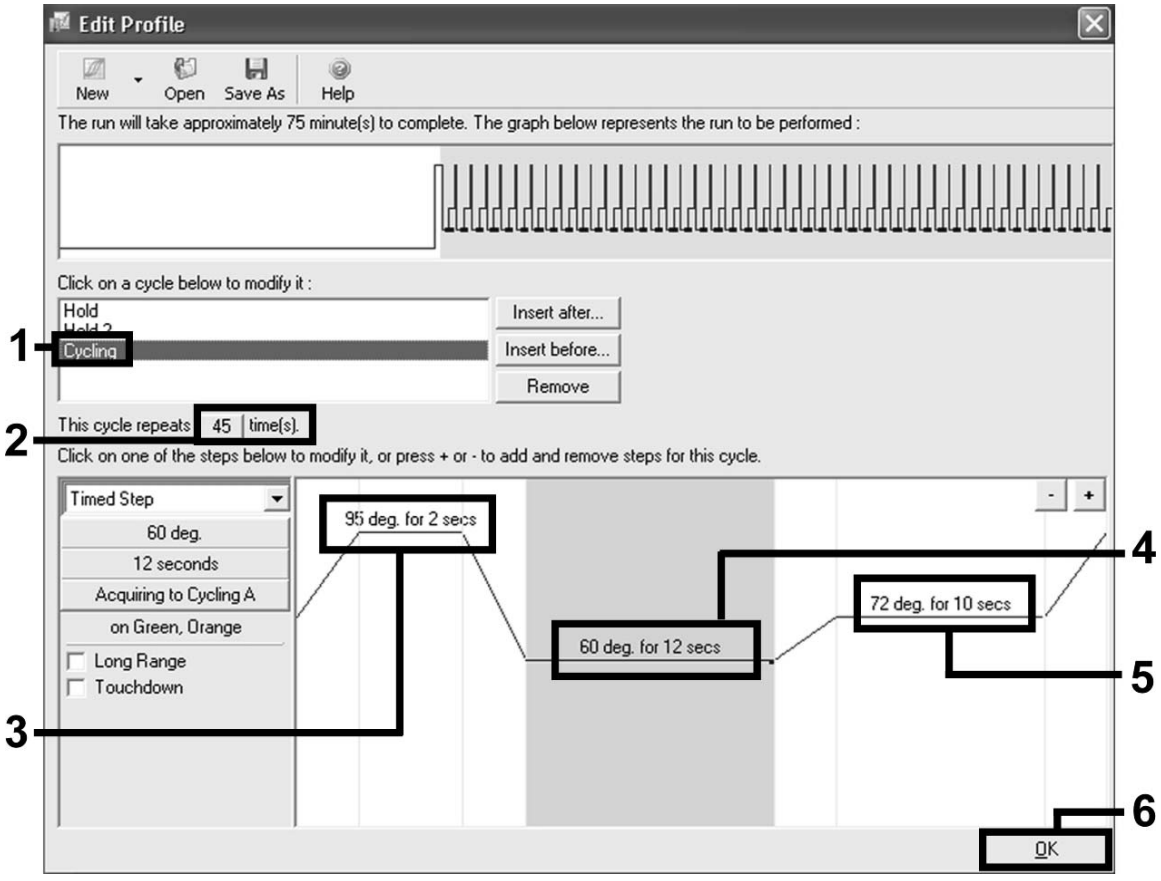

Figure 6. Amplification of the cDNA. Note that, on the Rotor-Gene 3000, the software will define the fluorescence dyes as "FAM/Sybr, ROX".

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. Check the box "Perform Optimisation before 1st Acquisition" (Figure 7). In the "New Run Wizard" dialog box, click "Next" (Figure 8).**

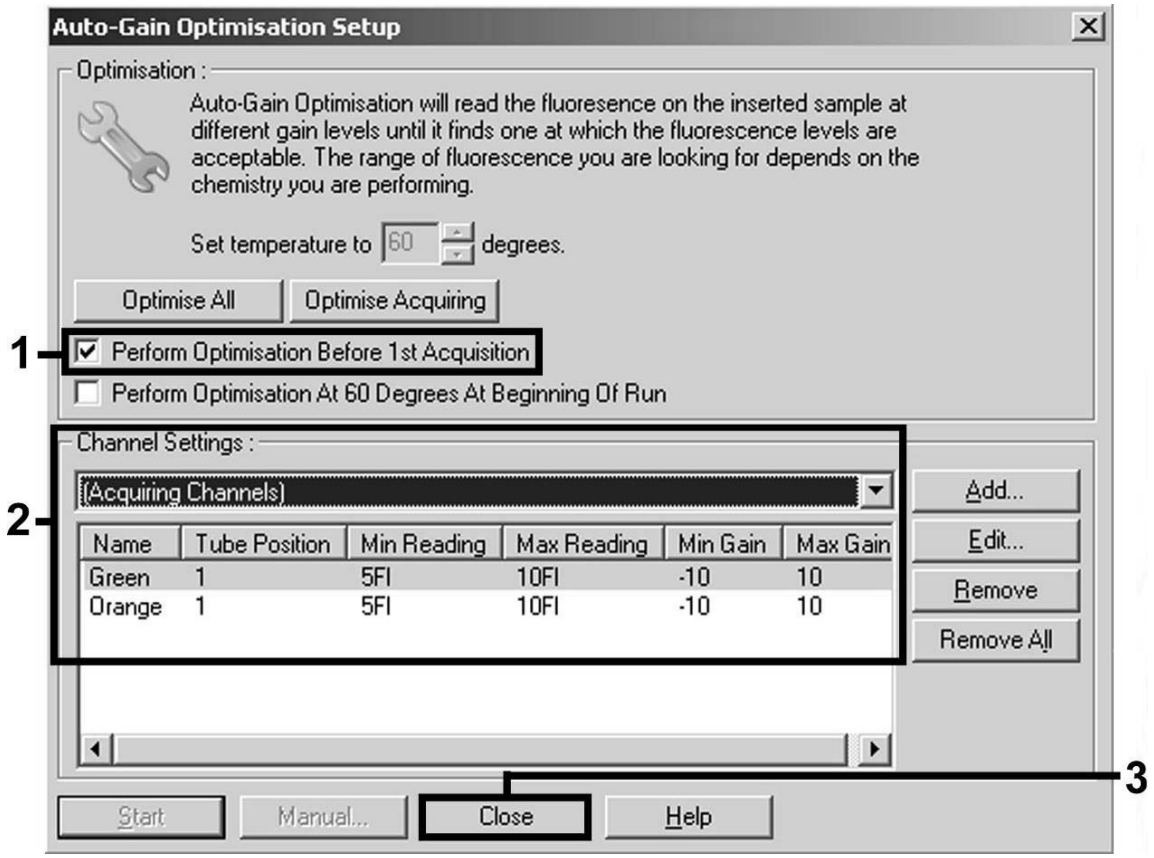

Figure 7. Adjusting the fluorescence channel sensitivity. Note that, on the Rotor-Gene 3000, the software will define the fluorescence dyes as "FAM/Sybr" and "ROX".

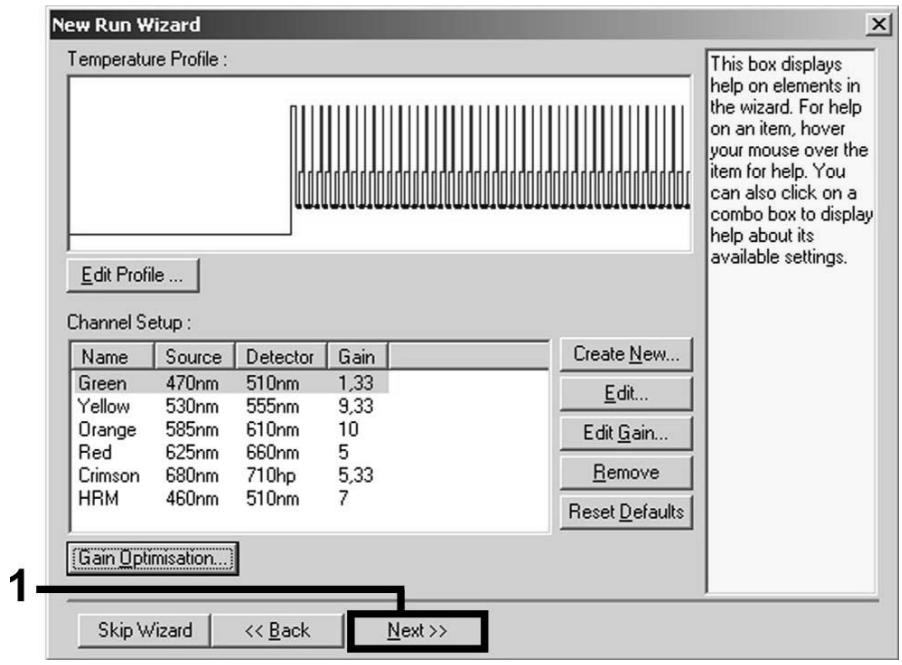

Figure 8. The "New Run Wizard" dialog box.

10. The gain values determined by the channel calibration are saved automatically and are listed in the last menu window of the programming procedure (Figure 9). Click "Start Run".

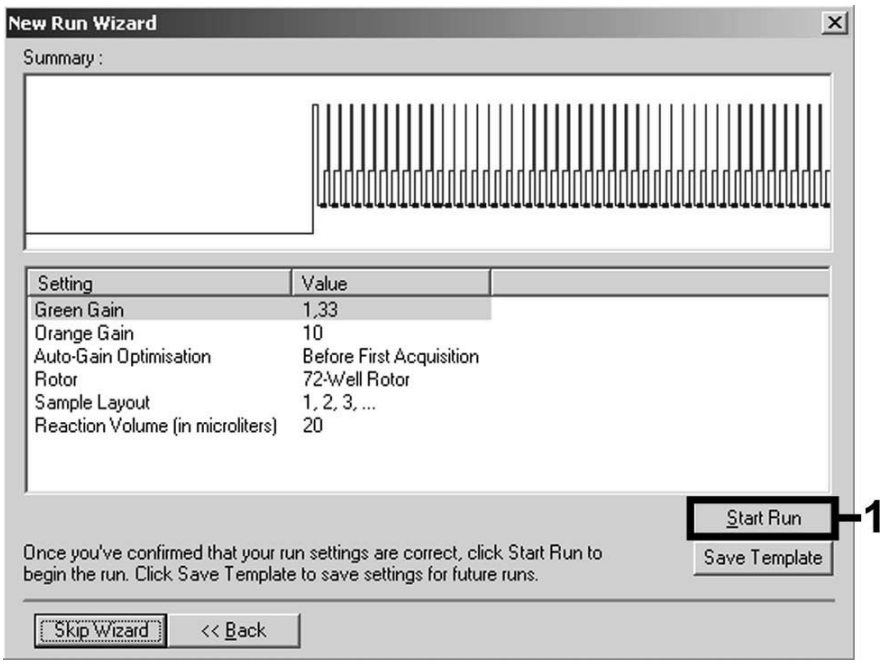

Figure 9. Starting the run. Note that, on the Rotor-Gene 3000, the software will define the fluorescence dyes as "FAM/Sybr" and "ROX".

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 for influenza virus in Figure 10 and Figure 11, and for influenza H1 virus in Figure 12.

11a. A signal is detected in fluorescence channel Cycling Green. The result of the analysis is positive: the sample contains influenza RNA or, for the Influenza H1 PCR, influenza H1 RNA.

In this case, the detection of a signal in the Cycling Orange channel is dispensable, since high initial concentrations of influenza RNA (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).

Note: On the Rotor-Gene 3000, the relevant channels are Cycling A.FAM for the positive signal and Cycling A.ROX for the internal control.

11b. In fluorescence channel Cycling Green no signal is detected. At the same time, a signal from the internal control appears in the Cycling Orange channel.
In the sample no influenza RNA is detectable. It can be considered negative.

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

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

Note: In some cases, the competitive IC PCR may reduce the signal of the analytical Influenza PCR. The Influenza H1 Master is therefore slightly more sensitive. We recommend testing samples with the Influenza Master and the Influenza H1 Master simultaneously in one run.

11c. No signal is detected in the Cycling Green or in the Cycling Orange channels.
Influenza PCR: No result can be concluded.
Influenza H1 PCR: If the same sample gives results described in 11a or 11b in the Influenza PCR, then in the sample no influenza H1 RNA is detectable. It can be considered negative. If both the Influenza PCR and the Influenza H1 PCR give results described in 11c, then no result can be concluded.

Information regarding error sources and their solution can be found in "Troubleshooting Guide", page 33.

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

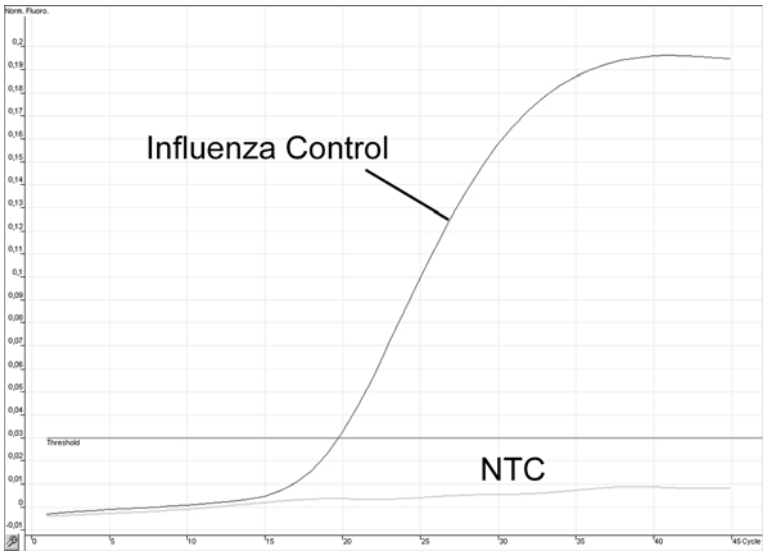

Figure 10. Detection of the Influenza Control in fluorescence channel Cycling Green. NTC: No template control (negative control).

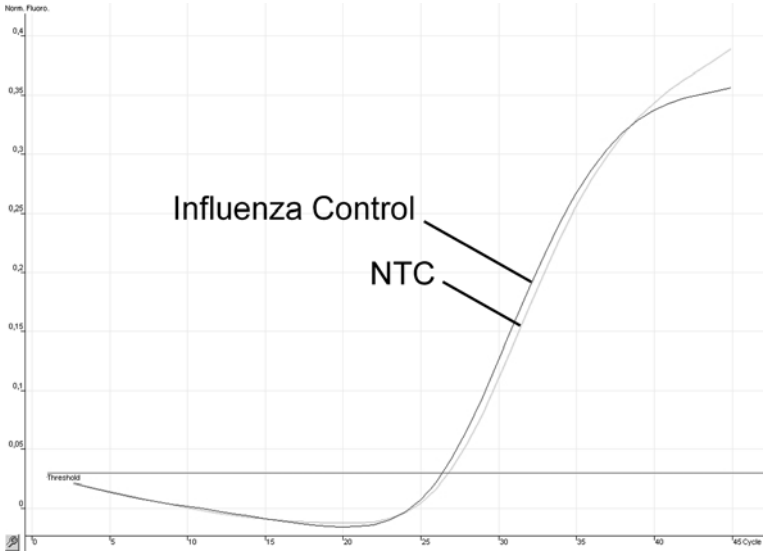

Figure 11. Detection of the internal control (IC) in fluorescence channel Cycling Orange with simultaneous amplification of the Influenza Control. NTC: No template control (negative control); amplification plot shows amplification of the internal control in a negative sample.

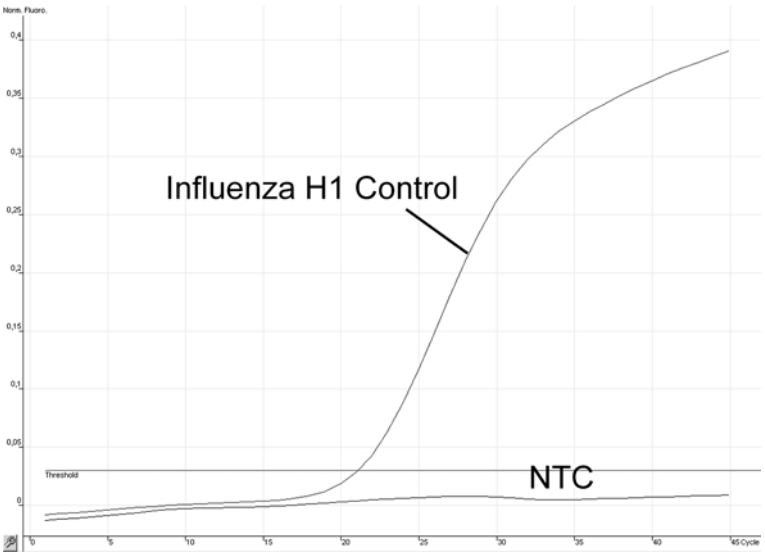

Figure 12. Detection of the Influenza H1 Control in fluorescence channel Cycling Green. NTC: No template control (negative control).

Protocol: PCR and Data Analysis (LightCycler Instruments)

Important points before starting

- Before beginning the procedure, read "Important Notes", pages 10–12.
- Take time to familiarize yourself with the LightCycler instrument before starting the protocol. See the instrument user manual.
- Make sure that the appropriate positive control (Influenza Control or Influenza H1 Control) as well as one no template control (Water, PCR grade) are included per PCR run.

Things to do before starting

- Make sure that the cooling block and the capillary adapters (accessories of the LightCycler Instrument) are 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 LightCycler capillaries into the adapters of the cooling block.

2. If you are using the internal control to monitor the RNA 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. For the Influenza H1 PCR, no internal control is included; follow step 2c.

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 6.

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

Table 6. Preparation of master mix (internal control used to monitor RNA isolation and check for PCR inhibition)

Number of samples	1	12
Influenza Master	12 µl	144 µl
Influenza Mg-Sol	3 µl	36 µl
Influenza IC	0 µl	0 µl
Total volume	**15 µl**	**180 µl**

2b. The internal control must be added directly to the mixture of Influenza Master and Influenza Mg-Sol. In this case, prepare a master mix according to Table 7.

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

Table 7. Preparation of master mix (internal control used exclusively to check for PCR inhibition)

Number of samples	1	12
Influenza Master	12 µl	144 µl
Influenza Mg-Sol	3 µl	36 µl
Influenza IC	0.5 µl	6 µl
Total volume	**15.5 µl***	**186 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.

2c. For the Influenza H1 PCR, no internal control is included. In this case, prepare a master mix according to Table 8.

Note: The Influenza H1 PCR should only be performed on samples that have tested positive for influenza virus RNA using the Influenza Master.

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

Table 8. Preparation of Influenza H1 master mix (no internal control)

Number of samples	1	12
Influenza H1 Master	12 µl	144 µl
Influenza Mg-Sol	3 µl	36 µl
Total volume	**15 µl**	**180 µl**

3. **Pipet 15 µl of the master mix into the plastic reservoir of each capillary (see Table 9). Correspondingly, 5 µl of the appropriate control (Influenza Control or Influenza H1 Control) must be used as a positive control and 5 µl of water (Water, PCR grade) as a no template control.**

Table 9. Preparation of PCR assay

Number of samples	1	12
Master mix	15 µl	15 µl each
Sample	5 µl	5 µl each
Total volume	**20 µl**	**20 µl each**

4. **Close the capillaries. To transfer the mixture from the plastic reservoir into the capillary, centrifuge the adapters containing the capillaries in a desktop centrifuge for 10 s at a maximum of 400 x *g* (2000 rpm).**
5. **For the detection of influenza virus RNA, create a temperature profile according to the following 4 steps (see Figures 13–16).**

Reverse transcription of the RNA	**Figure 13**
Initial activation of the hot-start enzyme	**Figure 14**
Amplification of the cDNA	**Figure 15**
Cooling	**Figure 16**

Note: Pay particular attention to the settings for Analysis Mode, Cycle Program Data, and Temperature Targets. In the illustrations these settings are framed in bold black.

All specifications refer to the LightCycler 1.1/1.2/1.5/2.0 instruments. Please find further information on programming LightCycler instruments in the instrument operator's manual. In the illustrations these settings are framed in bold black. Illustrations are included for LightCycler 1.1/1.2/1.5 instruments.

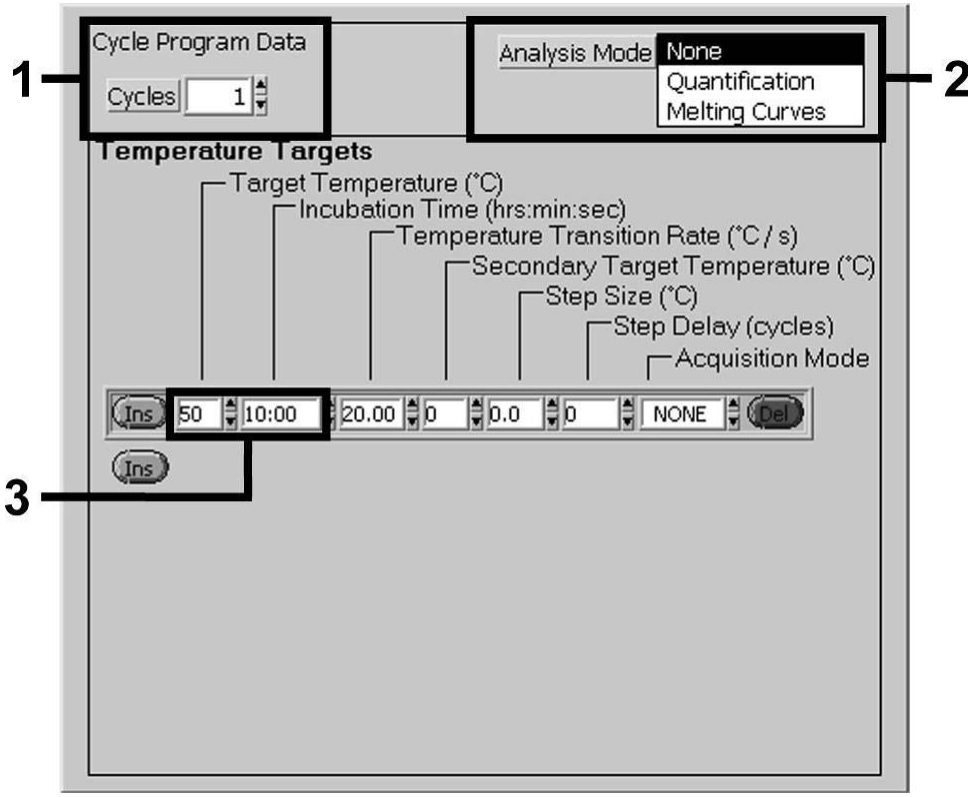

Figure 13. Reverse transcription of the RNA.

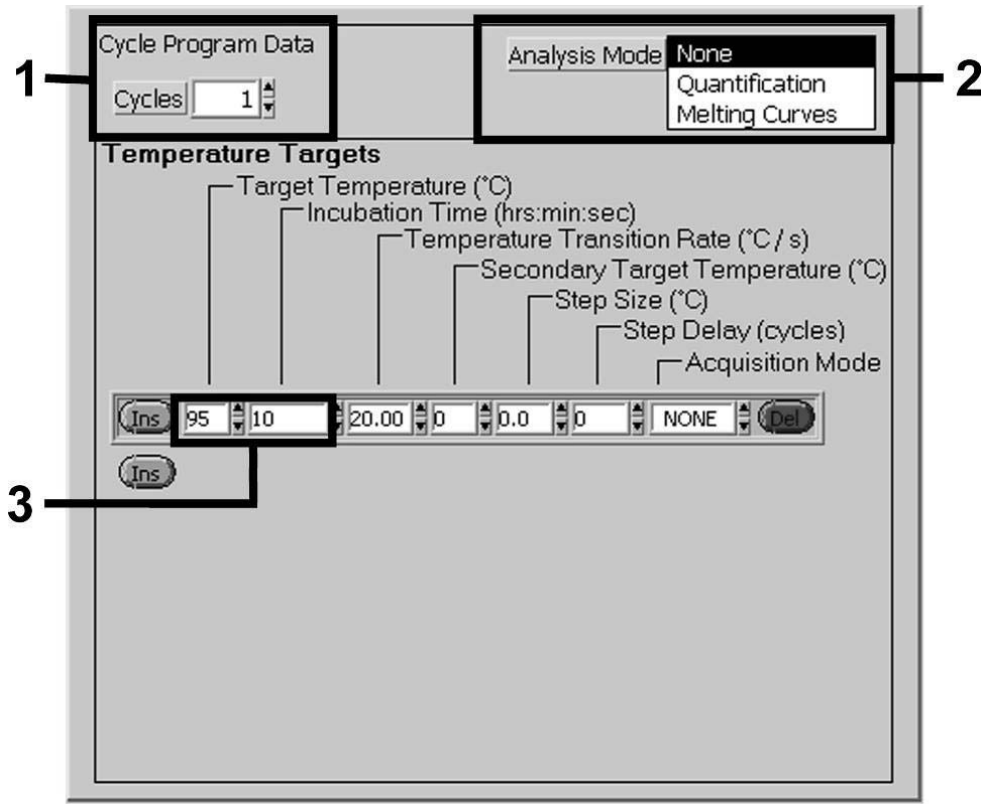

Figure 14. Initial activation of the hot-start enzyme.

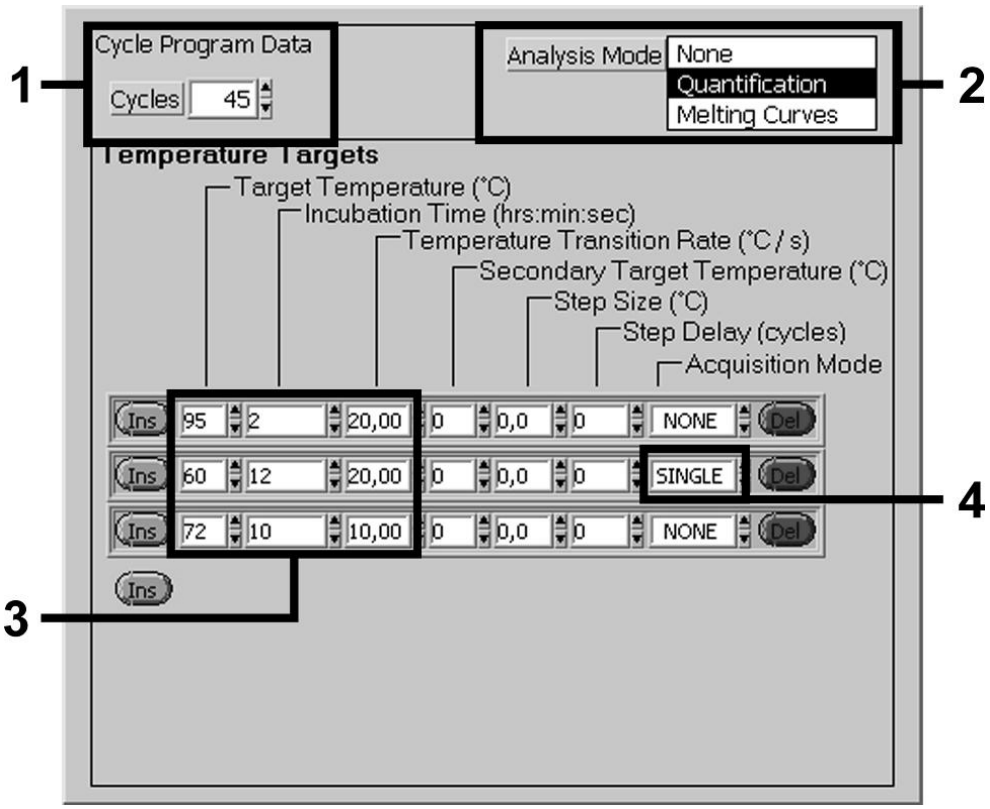

Figure 15. Amplification of the cDNA.

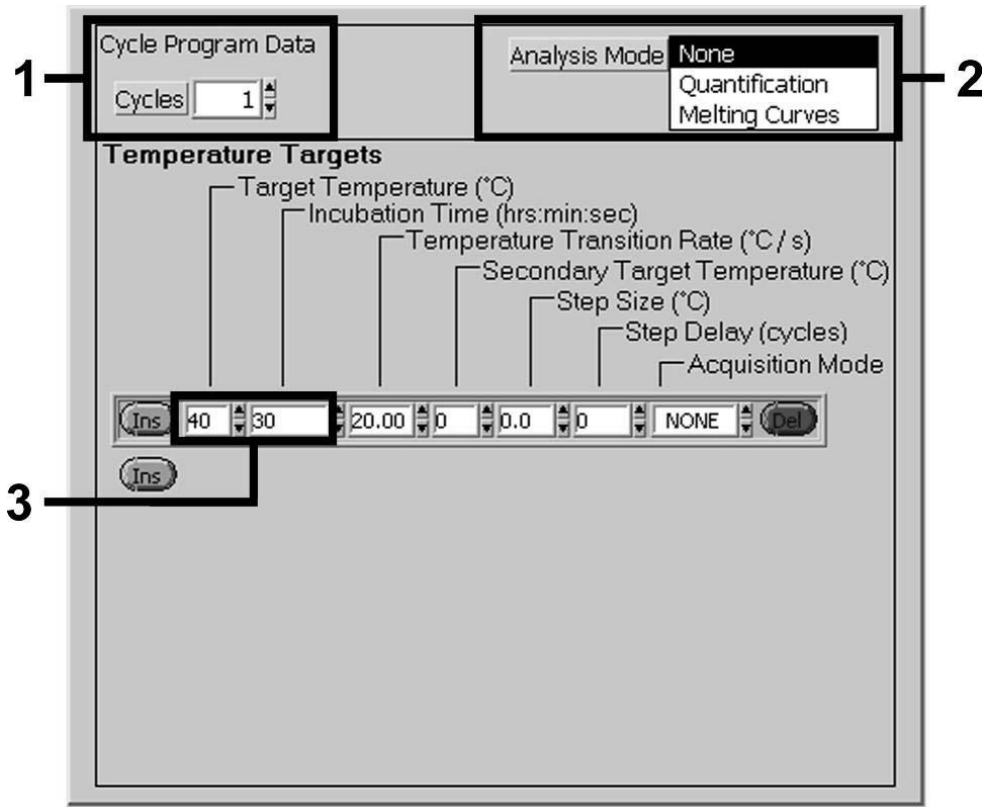

Figure 16. Cooling.

6. **Place the capillaries in the LightCycler instrument and start the cycling program**
7. **In multicolor analyses, interferences occur between fluorimeter channels. The LightCycler instrument's software contains a file termed "Color Compensation File", which compensates for these interferences. Open this file before, during, or after the PCR run by clicking the "Choose CCC File" or the "Select CC Data" buttons.**

   Note: If no **Color Compensation File** is installed, generate the file according to the instructions in the instrument operator's manual.

   After the **Color Compensation File** has been activated, separate signals appear in channels F1 and F2 (LightCycler 1.1/1.2/1.5 instruments) or fluorescence channels 530 and 610 (LightCycler 2.0 instrument).

8. The following results (8a, 8b, or 8c) are possible.

Examples of positive and negative PCR reactions are given for influenza virus in Figure 17 and Figure 18, and for influenza H1 virus in Figure 19.

8a. A signal is detected in channel F1 or 530.
The result of the analysis is positive: the sample contains influenza RNA or, for the Influenza H1 PCR, influenza H1 RNA.

In this case, the detection of a signal in the F2 or 610 channel is dispensable, since high initial concentrations of influenza RNA (positive signal in the F1 or 530 channel) can lead to a reduced or absent fluorescence signal of the internal control in the F2 or 610 channel (competition).

8b. In channel F1 or 530 no signal is detected. At the same time, a signal from the internal control appears in the F2 or 610 channel.
In the sample no influenza RNA is detectable. It can be considered negative.

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

Note: In some cases, the competitive IC PCR may reduce the signal of the analytical Influenza PCR. The Influenza H1 Master is therefore slightly more sensitive. We recommend to test samples with the Influenza Master and the Influenza H1 Master simultaneously in one run.

8c. No signal is detected in the F1 or 530 channel nor in the F2 or 610 channel.
Influenza PCR: No result can be concluded.
Influenza H1 PCR: If the same sample gives results described in 8a or 8b in the Influenza PCR, then in the sample no influenza H1 RNA is detectable. It can be considered negative. If both the Influenza PCR and the Influenza H1 PCR give results described in 8c, then no result can be concluded.

Information regarding error sources and their solution can be found in "Troubleshooting Guide", page 33.

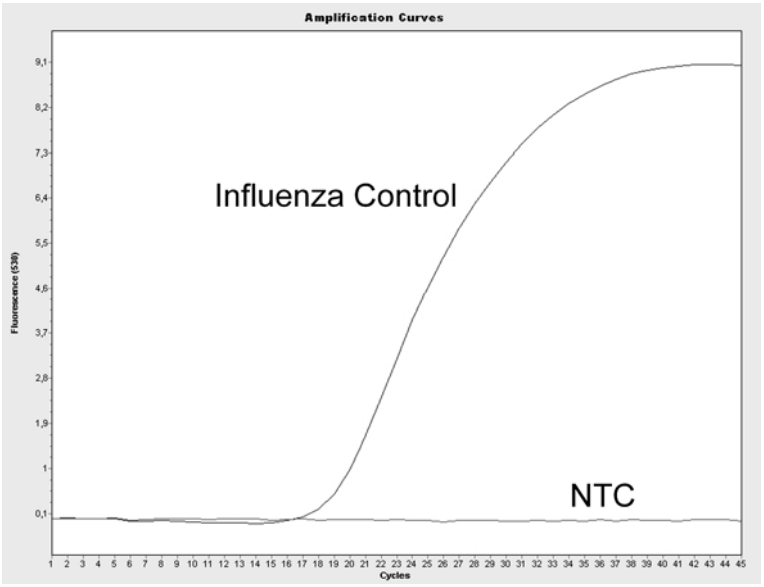

Figure 17. Detection of the Influenza Control in channel F1. On the LightCycler 2.0 instrument, the Influenza Control is detected in fluorescence channel 530. **NTC**: No template control (negative control).

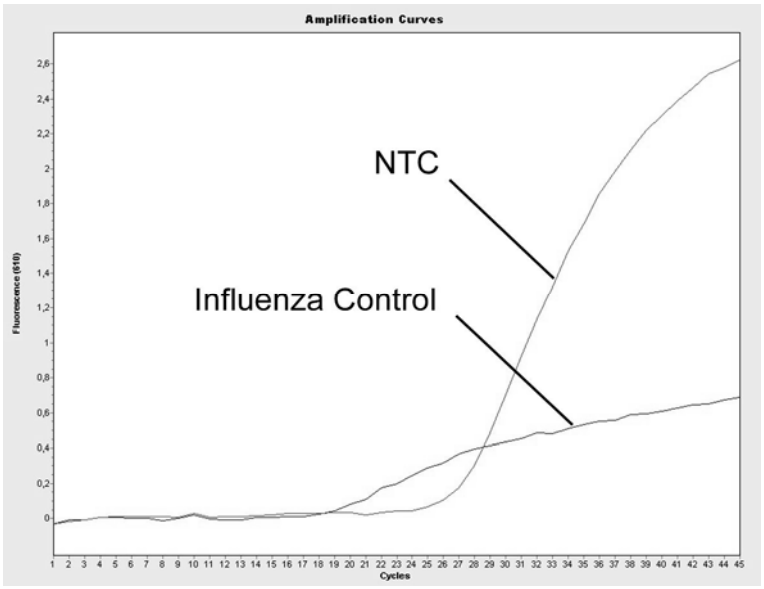

Figure 18. Detection of the internal control (IC) in channel F2 with simultaneous amplification of the Influenza Control. On the LightCycler 2.0 instrument, the internal control (IC) is detected in fluorescence channel 610. **NTC**: No template control (negative control) ; amplification plot shows amplification of the internal control in a negative sample.

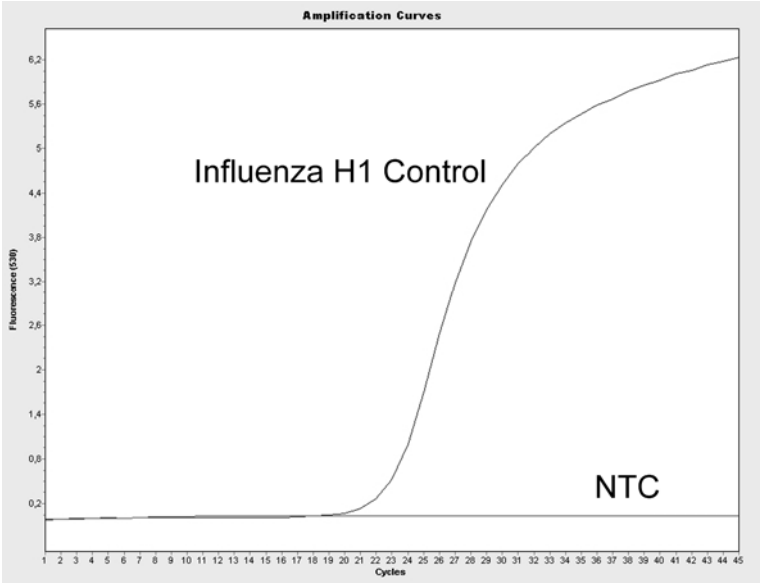

Figure 19. Detection of the Influenza H1 Control in channel F1. On the LightCycler 2.0 instrument, the Influenza H1 Control is detected in fluorescence channel 530. **NTC**: No template control (negative control).

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 (Influenza Control or Influenza H1 Control) in fluorescence channel Cycling Green, Cycling A.FAM, F1, or 530	
a) The selected fluorescence channel for PCR data analysis does not comply with the protocol	For data analysis select the fluorescence channel Cycling Green, Cycling A.FAM, F1, or 530 for the analytical Influenza and Influenza H1 PCR. Select the fluorescence channel Cycling Orange, Cycling A.ROX, F2, or 610 for the internal control RT-PCR.
b) Incorrect programming of the temperature profile of the instrument	Compare the temperature profile with the protocol. See "Protocol: PCR and Data Analysis (Rotor-Gene Instruments)", page 13, or "Protocol: PCR and Data Analysis (LightCycler Instruments)", page 24.
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 (Rotor-Gene Instruments)", page 13, or "Protocol: PCR and Data Analysis (LightCycler Instruments)", page 24.
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* Infl./H1 LC/RG RT-PCR Kit has expired	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.

	Comments and suggestions
Weak or no signal of the internal control in fluorescence channel Cycling Orange, Cycling A.ROX, F2, or 610 with simultaneous absence of a signal in channel Cycling Green, Cycling A.FAM, F1, or 530	
a) The PCR conditions do not comply with the protocol	Check the PCR conditions (see above) and repeat the PCR with corrected settings, if necessary.
b) The PCR was inhibited	Make sure that you use the recommended isolation method and closely follow the manufacturer's instructions.
c) RNA 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 RNA during the extraction. Make sure that you use the recommended isolation method (see "RNA 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* Infl./H1 LC/RG RT-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.
f) Influenza H1 PCR	The Influenza H1 reaction mix does not contain an internal control. No signal is expected in fluorescence channel Cycling Orange, Cycling A.ROX, F2, or 610.

Comments and suggestions	
Signals with the negative controls in fluorescence channel Cycling Green, Cycling A.FAM, F1, or 530 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 Infl./H1 LC/RG RT-PCR Kit (24)	For 24 reactions: 2 Masters (Influenza and Influenza H1), Mg Solution, 2 Positive Controls (Influenza and Influenza H1), Internal Control, Water (PCR grade)	4523003
artus Infl./H1 LC/RG RT-PCR Kit (96)	For 96 reactions: 2 Masters (Influenza and Influenza H1), Mg Solution, 2 Positive Controls (Influenza and Influenza H1), Internal Control, Water (PCR grade)	4523005
QIAamp Viral RNA Mini Kit — for purification of viral RNA from cell-free body fluids		
QIAamp Viral RNA Mini Kit (50)	For 50 preps: 50 QIAamp Mini Spin Columns, Carrier RNA, Collection Tubes (2 ml), RNase-Free Buffers	52904
QIAamp MinElute Virus Spin Kit — for simultaneous purification of viral DNA and RNA from plasma, serum, and cell-free body fluids using spin processing		
QIAamp MinElute Virus Spin Kit (50)	For 50 preps: 50 QIAamp MinElute Columns, QIAGEN Protease, Carrier RNA, Buffers, Collection Tubes (2 ml)	57704
EZ1 DSP Virus Kit — for automated, simultaneous purification of viral DNA and RNA from 1–6 human plasma, serum, or CSF samples 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	For in vitro diagnostic use in Europe: Robotic workstation for automated purification of nucleic acids using EZ1 DSP Kits, 1-year warranty on parts and labor	9001411

Product	Contents	Cat. no.
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
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

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

The purchase of this product allows the purchaser to use it for the performance of diagnostic services for human in vitro diagnostics. No general patent or other license of any kind other than this specific right of use from purchase is granted hereby.

THE PURCHASE OF THIS PRODUCT GRANTS THE PURCHASER RIGHTS UNDER ONE OR MORE OF U.S. PATENT NOS 6,174,670, 7,160,998, 6,569,627, 6,245,514, 5,804,375, 5,210,015, 5,487,972, 6,214,979 AND 7,141,377 AND THEIR FOREIGN COUNTERPARTS TO USE THIS PRODUCT SOLELY FOR PROVIDING HUMAN AND ANIMAL IN VITRO DIAGNOSTIC SERVICES. NO GENERAL PATENT OR OTHER LICENSE OF ANY KIND OTHER THAN THIS SPECIFIC RIGHT OF USE FROM PURCHASE IS GRANTED HEREBY.

Limited License Agreement

Use of this product signifies the agreement of any purchaser or user of the *artus* Infl./H1 LC/RG RT-PCR Kit to the following terms:

1. The *artus* Infl./H1 LC/RG RT-PCR Kit may be used solely in accordance with the artus *Infl./H1 LC/RG RT-PCR Kit Handbook* and for use with components contained in the Kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this Kit with any components not included within this Kit except as described in the artus *Infl./H1 LC/RG RT-PCR Kit Handbook* and additional protocols available at www.qiagen.com.
2. Other than expressly stated licenses, QIAGEN makes no warranty that this Kit and/or its use(s) do not infringe the rights of third-parties.
3. This Kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the Kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the Kit and/or its components.

For updated license terms, see www.qiagen.com.

www.qiagen.com

Australia ■ Orders 1-800-243-800 ■ Fax 03-9840-9888 ■ Technical 1-800-243-066

Austria ■ Orders 0800-28-10-10 ■ Fax 0800-28-10-19 ■ Technical 0800-28-10-11

Belgium ■ Orders 0800-79612 ■ Fax 0800-79611 ■ Technical 0800-79556

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 86-21-3865-3865 ■ Fax 86-21-3865-3965 ■ Technical 800-988-0325

Denmark ■ Orders 80-885945 ■ Fax 80-885944 ■ Technical 80-885942

Finland ■ Orders 0800-914416 ■ Fax 0800-914415 ■ Technical 0800-914413

France ■ Orders 01-60-920-926 ■ Fax 01-60-920-925 ■ Technical 01-60-920-930 ■ Offers 01-60-920-928

Germany ■ Orders 02103-29-12000 ■ Fax 02103-29-22000 ■ Technical 02103-29-12400

Hong Kong ■ Orders 800 933 965 ■ Fax 800 930 439 ■ Technical 800 930 425

Ireland ■ Orders 1800 555 049 ■ Fax 1800 555 048 ■ Technical 1800 555 061

Italy ■ Orders 800-789-544 ■ Fax 02-334304-826 ■ Technical 800-787980

Japan ■ Telephone 03-6890-7300 ■ Fax 03-5547-0818 ■ Technical 03-6890-7300

Korea (South) ■ Orders 080-000-7146 ■ Fax 02-2626-5703 ■ Technical 080-000-7145

Luxembourg ■ Orders 8002-2076 ■ Fax 8002-2073 ■ Technical 8002-2067

Mexico ■ Orders 01-800-7742-639 ■ Fax 01-800-1122-330 ■ Technical 01-800-7742-436

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

Norway ■ Orders 800-18859 ■ Fax 800-18817 ■ Technical 800-18712

Singapore ■ Orders 1800-742-4362 ■ Fax 65-6854-8184 ■ Technical 1800-742-4368

Spain ■ Orders 91-630-7050 ■ Fax 91-630-5145 ■ Technical 91-630-7050

Sweden ■ Orders 020-790282 ■ Fax 020-790582 ■ Technical 020-798328

Switzerland ■ Orders 055-254-22-11 ■ Fax 055-254-22-13 ■ Technical 055-254-22-12

UK ■ Orders 01293-422-911 ■ Fax 01293-422-922 ■ Technical 01293-422-999

USA ■ Orders 800-426-8157 ■ Fax 800-718-2056 ■ Technical 800-DNA-PREP (800-362-7737)

1062779 03/2010

Sample & Assay Technologies