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artus® HSV-1/2 Quant RG PCR Kit Handbook

Σ 96

Version 1
For use with Rotor-Gene® Q instruments

IVD

CE

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Sample to Insight

QIAGEN

Contents

Intended Use

The *artus*® HSV-1/2 Quant RG PCR Kit (96) is an *in-vitro* diagnostic test, based on real-time PCR technology, for the simultaneous detection and quantification of Herpes Simplex Virus 1 (HSV-1) and Herpes Simplex Virus 2 (HSV-2) specific DNA.

Summary and Explanation

The *artus* HSV-1/2 Quant RG PCR Kit constitutes a ready-to-use system for the detection of HSV-1- and HSV-2-specific DNA using real-time PCR on Rotor-Gene Q instruments. The assay includes a heterologous amplification system (Internal Control) to identify possible PCR inhibition and to confirm the integrity of the kit reagents.

Pathogen information

Herpes simplex virus 1 (HSV-1) and herpes simplex virus 2 (HSV-2) are members of the family *Herpesviridae* and, along with VZV, are classified as *Alphaherpesvirinae*. HSV-1 and HSV-2 have a linear double-stranded DNA genome of approximately 150 kbp. HSV-1 and HSV-2 share over 80% nucleotide identity within their protein-coding region.

Herpes simplex virus infections occur worldwide with no seasonal distribution. The virus is spread by direct contact with virus in secretions. The prevalence of HSV-1 infection increases gradually from childhood, reaching 80% and more in later years, whereas the seroprevalence of HSV-2 remains low until adolescence. Most HSV-1 primary infections are acquired as subclinical or unrecognized infections. Primary infections with HSV-2 classically present as herpes genitalis. Primary infection with HSV-1 or HSV-2 is followed by the establishment of latency in the dorsal root ganglia. Periodically, the virus reactivates and travels via the nerve axon to oral or genital sites, resulting in release of infectious virus and, in some cases, lesion formation. Although usually asymptomatic, HSV infections can cause a

wide spectrum of clinical manifestations, including oral herpes, genital herpes, neonatal herpes, encephalitis and ocular herpes.

Principle of the Procedure

The HSV-1/2 RG Master A and HSV-1/2 RG Master B contain reagents and enzymes for the specific amplification of target regions within the HSV-1 and HSV-2 genomes and for the direct detection of the specific amplicon in fluorescence channels Cycling Green and Cycling Red of Rotor-Gene Q instruments.

In addition, the *artus* HSV-1/2 Quant RG PCR Kit contains a heterologous amplification system to identify potential failures during the assay process. This is detected as an Internal Control (IC) in fluorescence channel Cycling Yellow of Rotor-Gene Q instruments.

Probes specific for HSV-1 DNA are labeled with the fluorophore FAM™, while probes specific for HSV-2 DNA are labeled with a fluorophore that displays the same characteristics as Cy®5. The probe specific for the Internal Control (IC) is labeled with the fluorophore JOE™. The use of probes labeled with spectrally distinguishable fluorophores enables simultaneous detection and quantification of HSV-1- and HSV-2-specific DNA as well as detection of the Internal Control in the corresponding channels of the Rotor-Gene Q instrument.

Materials Provided

Kit contents

***artus* HSV-1/2 Quant RG PCR Kit**		**(96)**
Catalog number		**4515265**
Number of reactions		**96**
Blue	HSV-1/2 RG Master A	8 x 60 µl
Purple	HSV-1/2 RG Master B	8 x 180 µl
Green	HSV-1/2 RG IC	1 x 1000 µl
Red	HSV-1 QS*	4 x 250 µl
Orange	HSV-2 QS*	4 x 250 µl
White	H_2O	1 x 500 µl
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* The *artus* HSV-1/2 Quant RG PCR Kit contains 4 HSV-1 Quantification Standards (QS1–QS4) as well as 4 HSV-2 Quantification Standards (QS1–QS4).

Materials Required but Not Provided

Prior to use, ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

Reagents

- QIAamp DNA Mini Kit (QIAGEN cat. no. 51304 or 51306; see "DNA extraction", page 10)

Consumables

- 0.1 ml Strip Tubes and Caps, for use with 72-well rotor (QIAGEN, cat. no. 981103 or 981106)
- Nuclease-free, low DNA-binding microcentrifuge tubes for preparing master mixes
- Nuclease-free pipet tips with aerosol barriers

Equipment

- Rotor-Gene Q MDx 5plex, Rotor-Gene Q 5plex or Rotor-Gene Q 6plex instrument
- Rotor-Gene Q software version 2.3.1 or higher
- Loading Block 72 x 0.1 ml Tubes, aluminum block for manual reaction setup (QIAGEN, cat. no. 9018901)
- Dedicated adjustable pipets for sample preparation
- Dedicated adjustable pipets for PCR master mix preparation
- Dedicated adjustable pipets for dispensing template DNA
- Vortex mixer
- Benchtop centrifuge with rotor for 2 ml reaction tubes

Warnings and Precautions

For in vitro diagnostic use.

Read all instructions carefully before using the test.

Warnings

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.

Precautions

- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.
- Specimens should always be treated as infectious and/or biohazardous in accordance with safe laboratory procedures.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.
- Avoid microbial and nuclease (DNase/RNase) contamination of the specimen and the components of the kit.
- Always use DNase/RNase-free disposable pipet tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.
- Use separated and segregated working areas for specimen preparation, reaction setup and amplification/detection activities. The workflow in the laboratory should proceed in a unidirectional manner. Always wear disposable gloves in each area, and change them before entering different areas.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separately from all other components of the kit.
- Do not open the reaction tubes/plates post amplification to avoid contamination with amplicons.
- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Do not use components of the kit that have passed their expiration date.
- Discard sample and assay waste according to your local safety regulations.

Reagent Storage and Handling

Kit components

The *artus* HSV-1/2 Quant RG PCR Kit is shipped on dry ice. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt, or if tubes have been compromised during shipment, contact QIAGEN Technical Services for assistance. Upon receipt, store all kit components at –30°C to –15°C.

Avoid thawing and freezing Master reagents (more than two times) as this may reduce assay performance. Freeze the reagents in aliquots if they are to be used intermittently. Do not store reagents at 4°C for longer than 2 hours. Protect HSV-1/2 RG Master A and HSV-1/2 RG Master B from light.

The *artus* HSV-1/2 Quant RG PCR Kit includes:

- Two Master reagents (HSV-1/2 RG Master A and HSV-1/2 RG Master B)
- Template Internal Control (HSV-1/2 RG IC)
- Four HSV-1 Quantification Standards (HSV-1 QS1–QS4)
- Four HSV-2 Quantification Standards (HSV-2 QS1–QS4)
- PCR-grade water (H_2O)

HSV-1/2 RG Master A and HSV-1/2 RG Master B reagents contain all components (buffer, enzymes, primers and probes) for the amplification, detection and differentiation of HSV-1- and HSV-2-specific DNA and the Internal Control in a single reaction.

The Quantification Standards contain standardized concentrations of HSV-1- and HSV-2-specific DNA. These can be used individually as positive controls or together to generate a standard curve, which can be used to determine the concentration of HSV-1- and/or HSV-2-specific DNA in the sample. The concentrations of the Quantification Standards are shown in Table 1.

Table 1. Concentration of Quantification Standards

	Concentration (copies/µl)	
Quantification Standard	**HSV-1**	**HSV-2**
QS1	10,000	10,000
QS2	1000	1000
QS3	100	100
QS4	10	10

Procedure

DNA extraction

HSV-1- and HSV-2-specific target sequences are amplified from DNA. As assay performance is dependent on the quality of the template DNA, make sure to use a sample preparation kit that yields DNA suitable for use in downstream PCR.

The QIAamp DNA Mini Kit (QIAGEN, cat. no. 51304 or 51306) is recommended for DNA purification for use with the *artus* HSV-1/2 Quant RG PCR Kit. Carry out DNA purification according to the instructions in the *QIAamp DNA Mini Handbook*.

As the wash buffers in the QIAamp DNA Mini Kit contain ethanol, carry out an additional centrifugation step prior to elution. Place the QIAamp Mini spin column in a new 2 ml collection tube and discard the old collection tube with the filtrate. Centrifuge for 10 minutes at approximately 17,000 x *g* (~13,000 rpm) in a benchtop centrifuge.

Important: The use of carrier RNA is crucial for extraction efficiency and stability of the extracted nucleic acid.

Important: Ethanol is a strong inhibitor in real-time PCR. If your sample preparation kit uses wash buffers containing ethanol, make sure to remove all traces of ethanol prior to elution of the nucleic acid.

Internal Control

The *artus* HSV-1/2 Quant RG PCR Kit contains a heterologous Internal Control, which can either be used as a PCR inhibition control or as a control of the sample preparation procedure (nucleic acid extraction) and as a PCR inhibition control.

If the Internal Control is used as a PCR inhibition control, but not as a control for the sample preparation procedure, add the Internal Control directly to the mixture of HSV-1/2 RG Master A and HSV-1/2 RG Master B, as described in step 2b of the protocol (page 13).

Regardless of which method/system is used for nucleic acid extraction, the Internal Control must not be added directly to the specimen. The Internal Control should always be added to the specimen/lysis buffer mixture. The volume of Internal Control to be added to the specimen/lysis buffer mixture depends only on the elution volume, and represents 10% of the elution volume. For example, when using the QIAamp DNA Mini Kit, the DNA is eluted in 60 µl Buffer AE. Therefore, add 6 µl Internal Control to the specimen/lysis buffer mixture of each sample.

Important: Do not add the Internal Control and/or the carrier RNA directly to the specimen.

Protocol: Detection of HSV-1- and HSV-2-specific DNA

Important points before starting

- Before beginning the procedure, read "Precautions", page 8.
- Take time to familiarize yourself with the Rotor-Gene Q instrument before starting the protocol. See the instrument user manual.
- Make sure that at least one positive control and one negative control (PCR-grade water) 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 samples, controls and Quantification Standards to be analyzed.

   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 2.

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

Table 2. Preparation of master mix (Internal Control used to monitor DNA isolation and to check for PCR inhibition)

Component	1 reaction	12 reactions
HSV-1/2 RG Master A	5 µl	60 µl
HSV-1/2 RG Master B	15 µl	180 µl
Total volume	**20 µl**	**240 µl**

2b. The Internal Control must be added directly to the mixture of HSV-1/2 RG Master A and HSV-1/2 Master B. In this case, prepare a master mix according to Table 3.

The reaction mix 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)

Component	1 reaction	12 reactions
HSV-1/2 RG Master A	5 µl	60 µl
HSV-1/2 RG Master B	15 µl	180 µl
HSV-1/2 RG IC	1 µl	12 µl
Total volume	**21 µl**	**252 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 20 µl of the master mix into each PCR tube. Then add 10 µl of the eluted sample DNA and mix well by pipetting repeatedly up and down. Correspondingly, add 10 µl of a positive control or Quantification Standard or 10 µl H_2O (PCR-grade water) as a negative control.

   Make sure to have at least one positive control and one negative control per run. For quantification, use all 8 Quantification Standards (HSV1 QS1–QS4 & HSV2 QS1–QS4).

4. Close the PCR tubes. Make sure that the locking ring (accessory of the Rotor-Gene instrument) is placed on top of the rotor.

5. For the detection of HSV-1- and HSV-2-specific DNA, create a temperature profile according to the following steps.

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

All specifications refer to the Rotor-Gene Q software version 2.3.1, 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.

6. First, open the **New Run Wizard** dialog box with the **Advanced** version and select **Two Step** (Figure 1). Click **Next** to continue.

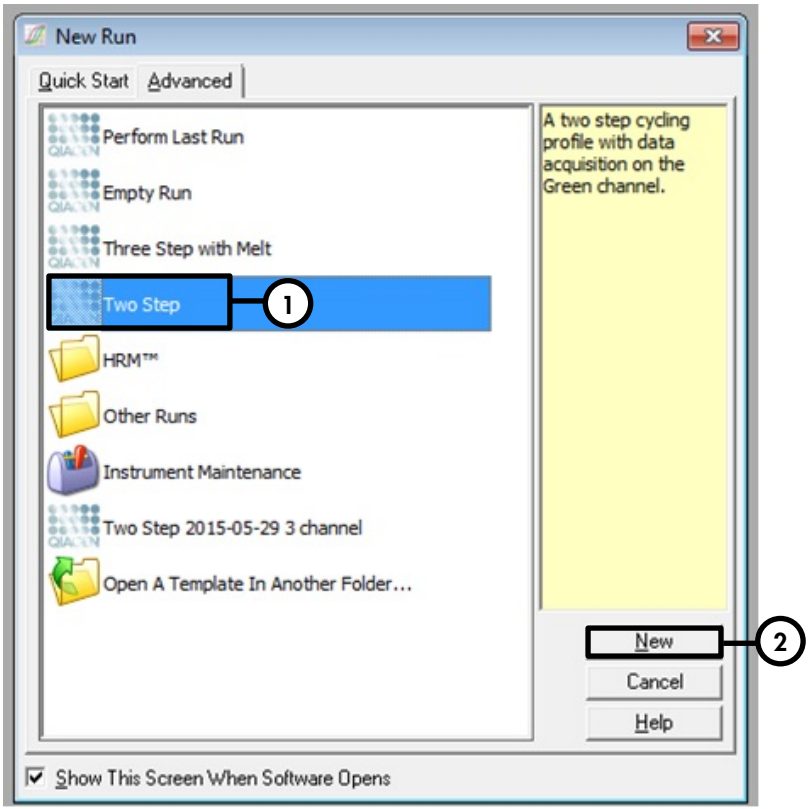

Figure 1. The New Run dialog box.

7. In the next **New Run Wizard** dialog box (Figure 2), check the **Locking Ring Attached** box and click **Next**.

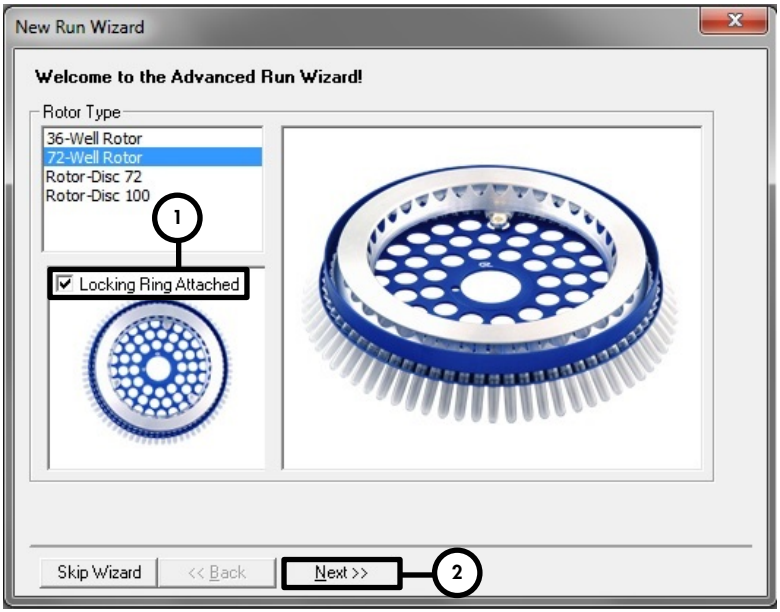

Figure 2. The New Run Wizard dialog box.

8. Select **30** for the PCR reaction volume and click **Next** (Figure 3).

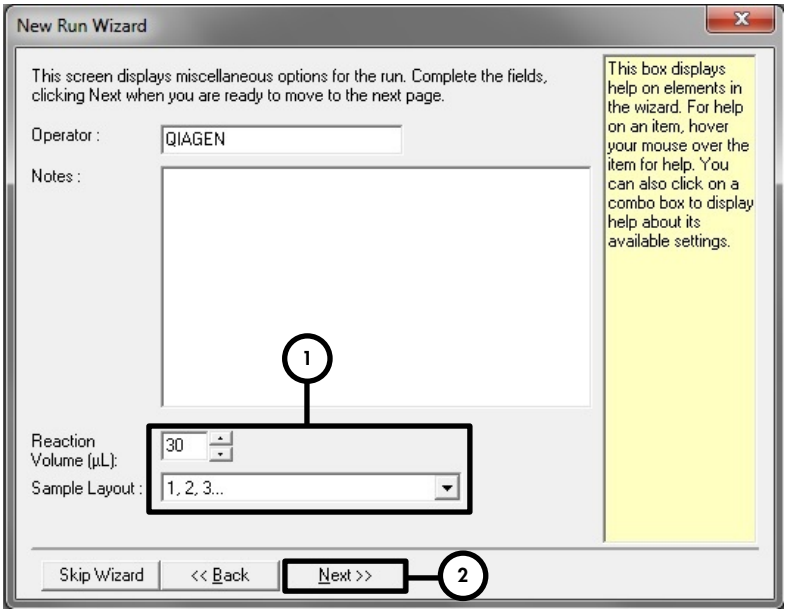

Figure 3. Setting the general assay parameters.

9. Click the **Edit Profile** button in the next **New Run Wizard** dialog box (Figure 4), and program the temperature profile as shown in Figures 5–6.

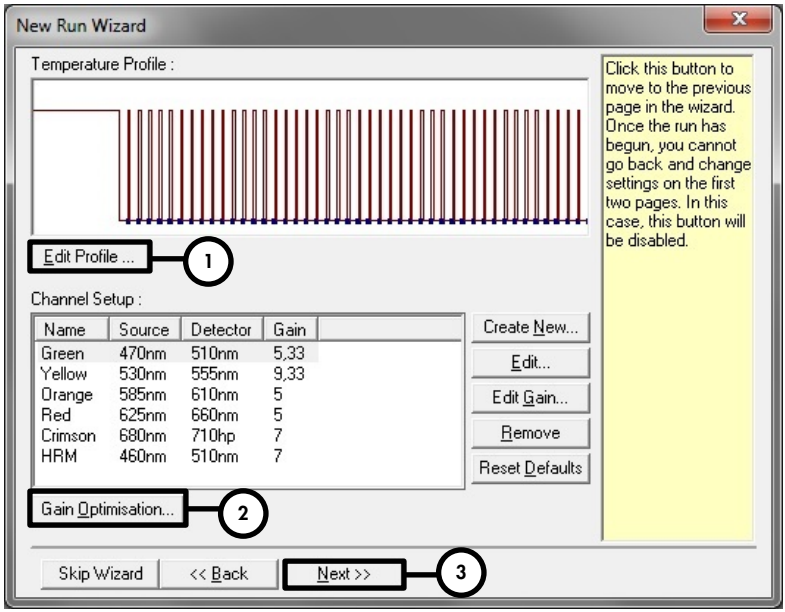

Figure 4. Editing the profile.

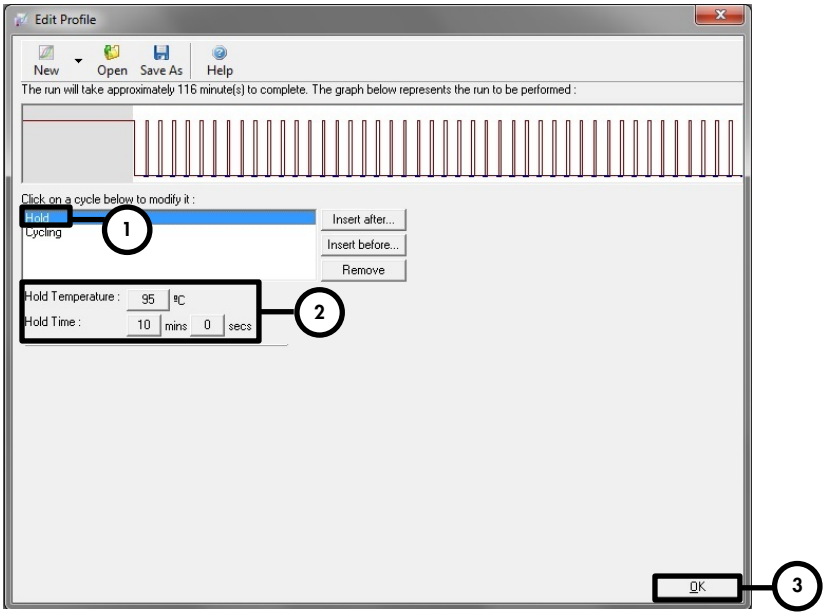

Figure 5. Initial activation of the hot-start enzyme.

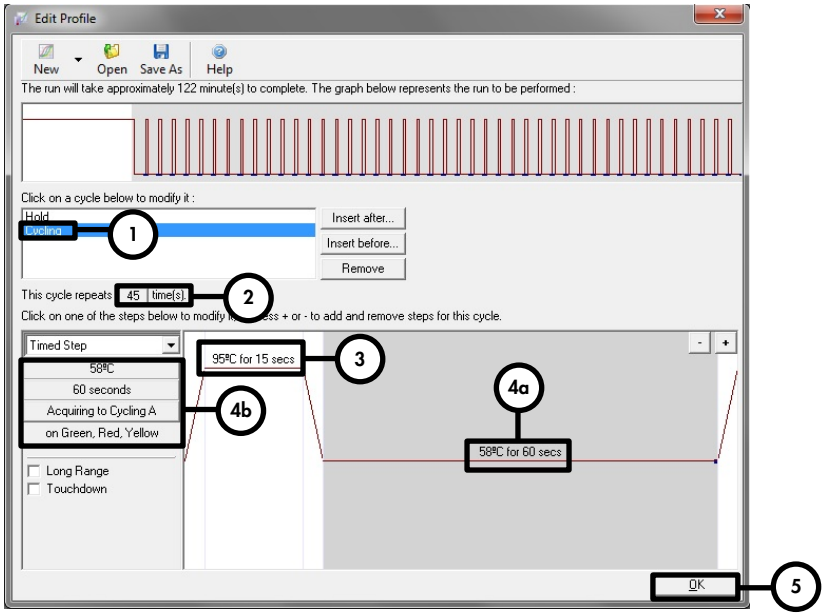

Figure 6. Amplification of the DNA.

10. The detection range of the fluorescence channels has to be determined according to the fluorescence intensities in the PCR tubes. Click **Gain Optimisation** in the **New Run Wizard** dialog box (see Figure 4, Step 2) to open the **Auto-Gain Optimisation Setup** dialog box (Figure 7). Check the **Perform Optimisation Before 1st Acquisition** box (Figure 7). Make sure that all three channels (Green, Red and Yellow) are selected for **Auto-Gain Optimisation** (Figure 7). (Find channels in the drop down menu under **Channel Settings** and click **Add**.) Click **Close** of the **Auto-Gain Optimisation Setup** dialog box when the gain calibration is completed.

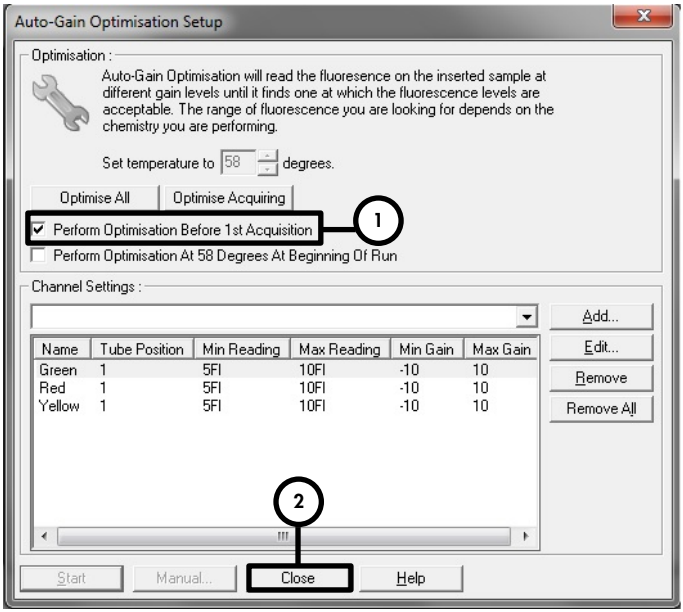

Figure 7. Adjusting the fluorescence channel sensitivity.

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

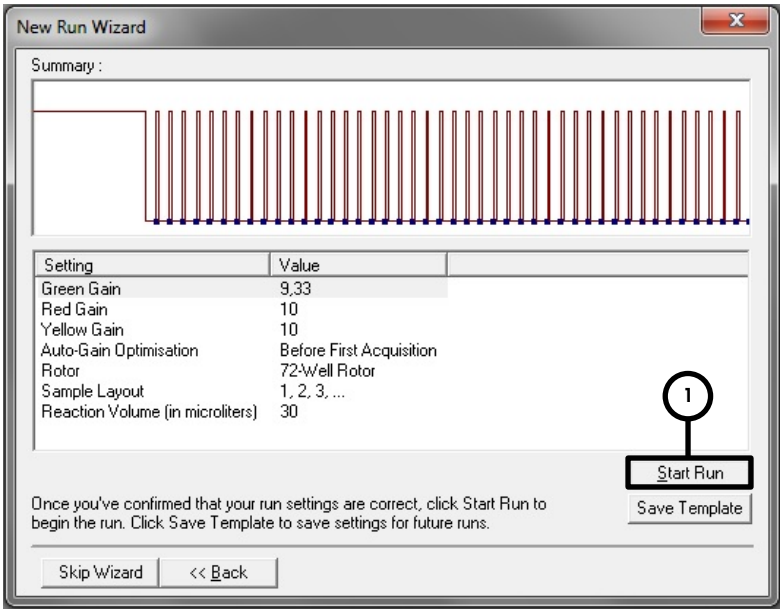

Figure 8. Starting the run.

12. After the run is finished, analyze the data (see "Interpretation of Results", page 23).

Interpretation of Results

Run validity

Valid qualitative run

The following control conditions must be met for a qualitative run to be valid (Table 4).

Table 4. Control conditions for a valid qualitative run

	Detection channel		
Control ID	**Cycling Green**	**Cycling Red**	**Cycling Yellow**
HSV-1 positive control (QS)	POSITIVE	NEGATIVE	POSITIVE
HSV-2 positive control (QS)	NEGATIVE	POSITIVE	POSITIVE
Negative control	NEGATIVE	NEGATIVE	POSITIVE

Invalid qualitative run

A qualitative run is invalid if the run has not been completed or if any of the control conditions for a valid qualitative run have not been met.

In case of an invalid qualitative run, repeat the PCR or extract DNA from the original samples again if no DNA is left over.

Valid quantitative run

A quantitative run is valid if all control conditions for a valid qualitative run have been met (see Table 4, above). Furthermore, for accurate quantification results, a valid standard curve needs to be generated. For a valid quantitative run, the standard curve must have the following control parameter values (Table 5).

Table 5. Control parameters for a valid standard curve

Control parameter	Valid value
Slope	–3.743/–2.765
PCR efficiency	85%/130%
R squared (R^2)	>0.98

Invalid quantitative run

A quantitative run is invalid if the run has not been completed or if any of the control conditions for a valid quantitative run have not been met.

In case of an invalid quantitative run, repeat the PCR or extract DNA from the original samples again if no DNA is left over.

Qualitative analysis

A summary of results interpretation is shown in Table 6.

Table 6. Summary of results interpretation

Sample ID	Detection channel			Result interpretation
	Cycling Green	Cycling Red	Cycling Yellow	
A	POSITIVE	NEGATIVE	POSITIVE*	HSV-1-specific DNA detected.
B	NEGATIVE	POSITIVE	POSITIVE*	HSV-2-specific DNA detected.
C	NEGATIVE	NEGATIVE	POSITIVE	Neither HSV-1- nor HSV-2-specific DNA detected. Sample does not contain detectable amounts of HSV-1- or HSV-2-specific DNA.
D	NEGATIVE	NEGATIVE	NEGATIVE	PCR inhibition or reagent failure. Repeat procedure using original sample or collect and test a new sample.

* Detection of the Internal Control in the Cycling Yellow channel is not required for positive results either in the Cycling Green detection channel or in the Cycling Red detection channel. High HSV-1 or HSV-2 loads in the sample can lead to reduced or absent Internal Control signals.

Quantitative analysis

The *artus* HSV-1/2 Quant RG PCR Kit contains 4 Quantification Standards (QS) for HSV-1 and 4 Quantification Standards (QS) for HSV-2. To generate a standard curve for quantitative analysis, these have to be defined as standards with appropriate concentrations (see Table 1, page 10). A standard curve for quantitative analysis can be generated using standards of known concentrations.

$C_T = m \log(N_0) + b$

- C_T = Threshold cycle
- m = Slope
- N_0 = Initial concentration
- b = Intercept

The concentrations of positive samples of unknown concentration can be derived from the standard curve (Figure 9).

$N_0 = 10^{(C_T-b)/m}$

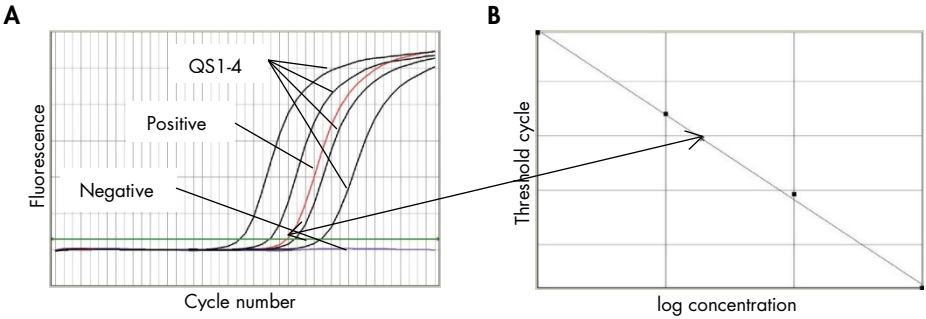

Figure 9. Quantification Standards, a positive and a negative sample displayed in (A) an amplification plot and (B) standard curve analysis.

Note: The concentration of the sample is displayed in copies/µl and refers to the concentration of viral DNA in the eluate.

Use the following formula to determine the viral load of the original sample.

$$\text{Viral load (sample) [copies/ml]} = \frac{\text{Volume (eluate) [µl] x viral load (eluate) [copies/µl]}}{\text{Sample input [ml]}}$$

Limitations

- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.
- Good laboratory practice is essential for proper performance of this assay.
- Take extreme care to preserve the purity of the components of the kit and reaction setups. Closely monitor all reagents for impurities and contamination. Discard any reagents suspected of contamination.
- Appropriate specimen collection, transport, storage and processing procedures are required for optimal performance of this assay.
- Do not use this assay directly on the specimen. Perform the applicable nucleic acid extraction procedures prior to using this assay.
- The presence of PCR inhibitors may cause false-negative or invalid results.
- Potential mutations within the target regions of the HSV-1 and/or HSV-2 genome covered by the primers and/or probes used in the kit may result in failure to detect the presence of the pathogens.
- As with any diagnostic test, interpret the results obtained using the *artus* HSV-1/2 Quant RG PCR Kit in consideration of all clinical and laboratory findings.

Quality Control

Each lot of *artus* HSV-1/2 Quant RG PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Performance Characteristics

The specific performance characteristics of the *artus* HSV-1/2 Quant RG PCR Kit were determined using HSV-1-specific DNA (ATCC® number: VR-1493) and HSV-2-specific DNA (ATCC number: VR-540) of known concentrations.

Analytical sensitivity

The analytical sensitivity of the *artus* HSV-1/2 Quant RG PCR Kit is defined as the concentration (copies per µl of the eluate) of HSV-1- or HSV-2-specific DNA that can be detected with a positivity rate of ≥95%. The analytical sensitivity was determined by analysis of a dilution series of HSV-1 DNA and HSV-2 DNA of known concentration (Tables 7 and 8).

Table 7. PCR results used to calculate the analytical sensitivity of HSV-1-specific amplification

Input concentration (copies/µl)	Number of replicates	Number of positives	Hit rate (%)
3.16	12	12	100
1.0	12	12	100
0.32	12	11	91.6
0.1	12	9	75
0.03	12	6	50
0.01	12	2	16.7
0.003	12	0	0
0.001	12	0	0
NTC	12	0	0

Table 8. PCR results used to calculate the analytical sensitivity of HSV-2-specific amplification

Input concentration (copies/µl)	Number of replicates	Number of positives	Hit rate (%)
3.16	18	18	100
1.0	18	18	100
0.32	18	11	61.1
0.1	18	7	38.9
0.03	18	3	16.7
0.01	18	1	5.6
0.003	18	0	0
0.001	18	0	0
NTC	18	0	0

The analytical sensitivity of the *artus* HSV-1/2 Quant RG PCR Kit, determined by probit analysis, for detection of HSV-1-specific DNA is 0.33 copies/µl eluate (95% confidence interval [CI]: 0.16–1.3 copies/µl) and the analytical sensitivity for detection of HSV-2-specific DNA is 1.2 copies/µl eluate (95% CI: 0.7–3.5 copies/µl).

Analytical specificity

The analytical specificity of the *artus* HSV-1/2 Quant RG PCR Kit is ensured by careful selection of the oligonucleotides (primers and probes). The oligonucleotides are checked by sequence comparison analysis against publically available sequences to ensure that all relevant HSV genotypes are detected. In addition, the specificity of the *artus* HSV-1/2 Quant RG PCR Kit was evaluated by testing a panel of genomic DNA/RNA extracted from other herpesviruses or other pathogens that are relevant to immunocompromised patients (Table 9).

Table 9. Organisms tested for cross-reactivity

Organism	Detection channel		
	Cycling Green (HSV-1)	Cycling Red (HSV-2)	Cycling Yellow (IC)
Varicella-zoster virus	Negative	Negative	Valid
Epstein-Barr virus	Negative	Negative	Valid
Cytomegalovirus	Negative	Negative	Valid
Human herpesvirus 6 (A, B)	Negative	Negative	Valid
Human herpesvirus 7	Negative	Negative	Valid
Human herpesvirus 8	Negative	Negative	Valid
BK virus	Negative	Negative	Valid
JC virus	Negative	Negative	Valid
Parvovirus B19	Negative	Negative	Valid
Hepatitis A virus	Negative	Negative	Valid
Hepatitis B virus	Negative	Negative	Valid
Hepatitis C virus	Negative	Negative	Valid
Human immunodeficiency virus 1	Negative	Negative	Valid

The *artus* HSV-1/2 Quant RG PCR Kit did not cross-react with any of the specified organisms.

Linear range

The linear range of the *artus* HSV-1/2 Quant RG PCR Kit was evaluated by analyzing a logarithmic dilution series of HSV-1- and HSV-2-specific DNA using concentrations ranging from 10^8 copies/µl to 10 copies/µl (HSV-1) (Figure 10) and 10^7 to 10 copies/µl (HSV-2). At least 6 replicates per dilution were analyzed.

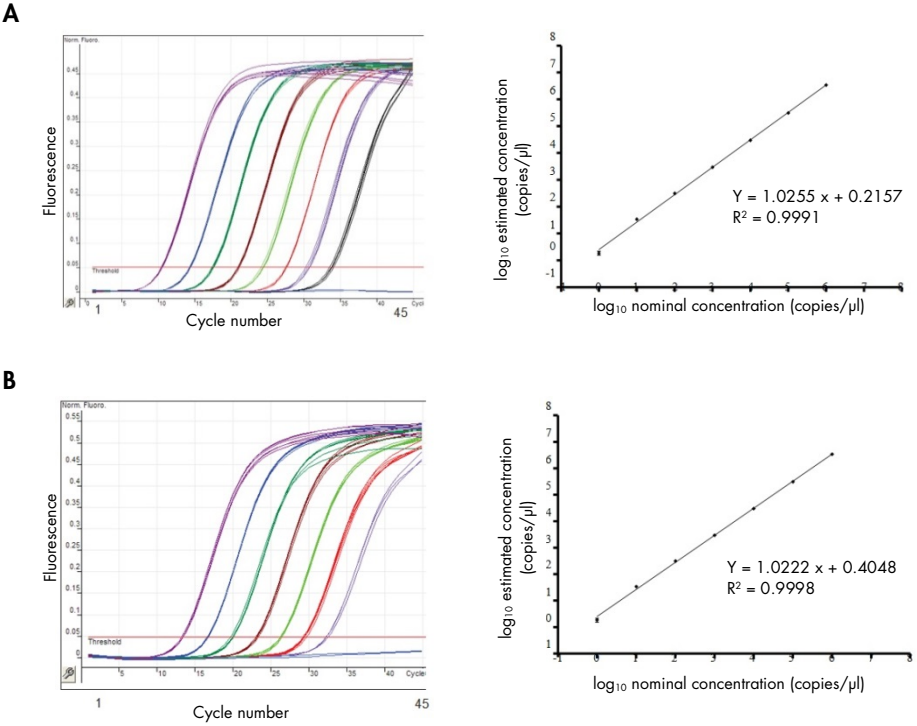

Figure 10. Amplification curves and linear regression analysis of a dilution series of (A) HSV-1- and (B) HSV-2-specific DNA.

The linear range of the *artus* HSV-1/2 Quant RG PCR Kit extends over an interval of at least 7 orders of magnitude for HSV-1- and over an interval of at least 6 orders of magnitude for HSV-2-specific DNA.

Precision

The precision of the *artus* HSV-1/2 Quant RG PCR Kit was determined as intra-assay variability (variability within one experiment), inter-assay variability (variability between different experiments) and inter-lot variability (variability between different production lots).

Variability data are expressed in terms of standard deviation, variance and coefficient of variation. The data are based on quantification analysis of defined concentrations of HSV-1- and HSV-2-specific DNA and on threshold cycle (C_T) values in terms of the Internal Control (Tables 10–13). At least 6 replicates per sample were analyzed for intra-assay, inter-assay and inter-lot variability. Total variance was calculated by combining the 3 analyses.

Table 10. Precision of amplification of HSV-1-specific DNA

HSV-1-specific system	Average conc. (copies/µl)	Standard deviation	Variance	Coefficient of variation (%)
Intra-assay variability	91	5.3	29	5.9
	8.8	1.5	2.2	16.7
Inter-assay variability	94.2	5.3	29.3	5.7
	8.9	1.2	1.4	13.1
Inter-lot variability	90.3	5.1	25.5	5.6
	8.7	1.2	1.5	14.2
Total variance	92.7	5.5	30.7	6.0
	8.8	1.1	1.2	12.7

Table 11. Precision of amplification of Internal Control for HSV-1

Internal Control	Average threshold cycle (C_T)	Standard deviation	Variance	Coefficient of variation (%)
Intra-assay variability	23.0	0.05	0.003	0.23
Inter-assay variability	22.9	0.12	0.01	0.51
Inter-lot variability	23.5	0.61	0.37	2.6
Total variance	23.3	0.61	0.37	2.6

Table 12. Precision of amplification of HSV-2-specific DNA

HSV-2-specific system	Average conc. (copies/µl)	Standard deviation	Variance	Coefficient of variation (%)
Intra-assay variability	108	5.9	35	5.5
	9.8	1.8	3.4	18.0
Inter-assay variability	99.2	9.4	87.7	9.4
	10	2.0	4.15	20.4
Inter-lot variability	102.5	9.5	90.8	9.3
	9.0	2.0	4.0	22.2
Total variance	99.6	9.0	81.7	9.1
	9.5	2.1	4.5	22.3

Table 13. Precision of amplification of Internal Control for HSV-2

Internal Control	Average threshold cycle (C_T)	Standard deviation	Variance	Coefficient of variation (%)
Intra-assay variability	24.0	0.1	0.004	0.43
Inter-assay variability	23.8	0.3	0.13	1.27
Inter-lot variability	24.0	0.14	0.02	0.59
Total variance	23.9	0.25	0.06	1.03

Repeatability

Specificity, sensitivity and accuracy of quantification of the *artus* HSV-1/2 Quant RG PCR Kit were evaluated by analyzing established proficiency panels for HSV. To ensure repeatability of the *artus* HSV-1/2 Quant RG PCR Kit, specificity and sensitivity are evaluated by

analyzing established proficiency panels for HSV-1 and HSV-2 as well as characterized diagnostic samples on a regular basis (An example is shown in Table 15).

Table 15. Results of the analysis of a proficiency panel for HSV (QCMD)

Proficiency panel			*artus* HSV-1/2 Quant RG PCR Kit		
Sample ID	Sample content	Expected conc. (copies/ml)	Detected conc. of HSV-1 (copies/ml)	Detected conc. of HSV-2 (copies/ml)	Internal Control
HSVDNA14-01	HSV-1	5408	2460	–	Valid
HSVDNA14-02	HSV negative	–	–	–	Valid
HSVDNA14-03	HSV-1	1135	855	–	Valid
HSVDNA14-04	HSV-1	213	44	–	Valid
HSVDNA14-05	HSV-1	12,794	8490	–	Valid
HSVDNA14-06	HSV-2	1982	–	1881	Valid
HSVDNA14-07	HSV-2	275	–	525	Valid
HSVDNA14-08	HSV-2	5023	–	11,370	Valid
HSVDNA14-09	HSV-1	341	70	–	Valid
HSVDNA14-10	VZV	–	–	–	Valid

Symbols

The symbols in the following table are used in these instructions for use.

Symbol	Symbol definition
	Contains sufficient for 96 tests
	In vitro diagnostic medical device
	Catalog number
	Lot number
	Temperature limitation
	Manufacturer

Symbol	Symbol definition
	Use by
	Material number
	Global Trade Item Number
	Consult instructions for use

Troubleshooting Guide

The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

Ordering Information

Product	Contents	Cat. no.
artus HSV-1/2 Quant RG PCR Kit (96)	For 96 reactions: Master A, Master B, 4 Quantification Standards HSV-1, 4 Quantification Standards HSV-2, Internal Control, H_2O (PCR grade water)	4515265
QIAamp DNA Mini Kit (50)	For 50 DNA preps: 50 QIAamp Mini Spin Columns, Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51304
QIAamp DNA Mini Kit (250)	For 250 DNA preps: 250 QIAamp Mini Spin Columns, Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51306
Rotor-Gene Q and accessories		
Rotor-Gene Q MDx 5plex System	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training	9002023
Rotor-Gene Q MDx 5plex Platform	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training not included	9002022

Product	Contents	Cat. no.
Rotor-Gene Q 5plex Priority Package Plus	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories: includes Priority Package with software, installation, training, 3-year warranty on parts and labor, and 3 preventive maintenance visits	9001866
Rotor-Gene Q 5plex Priority Package	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories: includes Priority Package with software, installation, training, 2-year warranty on parts and labor, and 2 preventive maintenance visits	9001865
Rotor-Gene Q 5plex System	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training	9001640
Rotor-Gene Q 5plex Platform	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training not included	9001570

Product	Contents	Cat. no.
Rotor-Gene Q 6plex Priority Package Plus	Real-time PCR instrument with 6 channels (blue, green, yellow, orange, red, crimson), including laptop computer, software, accessories: includes Priority Package with software, installation, training, 3-year warranty on parts and labor, and 3 preventive maintenance visits	9001870
Rotor-Gene Q 6plex Priority Package	Real-time PCR instrument with 6 channels (blue, green, yellow, orange, red, crimson), including laptop computer, software, accessories: includes Priority Package with software, installation, training, 2-year warranty on parts and labor, and 2 preventive maintenance visits	9001869
Rotor-Gene Q 6plex System	Real-time PCR instrument with 6 channels (blue, green, yellow, orange, red, crimson), including laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training	9001660
Rotor-Gene Q 6plex Platform	Real-time PCR instrument with 6 channels (blue, green, yellow, orange, red, crimson), including laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training not included	9001590

Product	Contents	Cat. no.
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
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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Limited License Agreement for *artus* HSV-1/2 Quant RG PCR Kit

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