February 2015

artus® HSV-1/2 LC PCR Kit Handbook

Σ 24 (catalog no. 4500063)

Σ 96 (catalog no. 4500065)

Quantitative in vitro Diagnostics

For use with the *LightCycler*® Instrument

Version 1

CE

IVD

REF 4500063, 4500065

1046888

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artus HSV-1/2 LC PCR Kit

For use with the *LightCycler* Instrument.

1. Contents

	Labelling and contents	Art. No. 4500063 24 reactions	Art. No. 4500065 96 reactions
Blue	*HSV LC Master*	2 x 12 rxns	8 x 12 rxns
Red	*HSV1 LC/RG/TM QS 1*¤ 1 x 10^4 cop/µl	1 x 200 µl	1 x 200 µl
Red	*HSV1 LC/RG/TM QS 2*¤ 1 x 10^3 cop/µl	1 x 200 µl	1 x 200 µl
Red	*HSV1 LC/RG/TM QS 3*¤ 1 x 10^2 cop/µl	1 x 200 µl	1 x 200 µl
Red	*HSV1 LC/RG/TM QS 4*¤ 1 x 10^1 cop/µl	1 x 200 µl	1 x 200 µl
Red	*HSV2 LC/RG/TM QS 1*¤ 1 x 10^4 cop/µl	1 x 200 µl	1 x 200 µl
Red	*HSV2 LC/RG/TM QS 2*¤ 1 x 10^3 cop/µl	1 x 200 µl	1 x 200 µl
Red	*HSV2 LC/RG/TM QS 3*¤ 1 x 10^2 cop/µl	1 x 200 µl	1 x 200 µl
Red	*HSV2 LC/RG/TM QS 4*¤ 1 x 10^1 cop/µl	1 x 200 µl	1 x 200 µl
Green¤	*HSV LC IC*	1 x 1,000 µl	2 x 1,000 µl
White	*Water (PCR grade)*	1 x 1,000 µl	1 x 1,000 µl

¤ *QS = Quantitation Standard*
IC = Internal Control

2. Storage

The components of the *artus* HSV-1/2 LC PCR Kit should be stored at –15°C to –30°C and are stable until the expiry date stated on the label. Repeated thawing and freezing (> 2 x) should be avoided, as this may reduce the sensitivity. If the reagents are to be used only intermittently, they should be frozen in aliquots. Storage at +4°C should not exceed a period of five hours.

3. Additionally Required Materials and Devices

- Disposable, powder-free gloves
- DNA isolation kit (see 8.1 DNA Isolation)
- Pipettes (adjustable)
- Sterile pipette tips with filters
- Vortex mixer
- Desktop centrifuge with rotor for 2 ml reaction tubes
- *Color Compensation Set* (Roche Diagnostics, Cat. No. 2 158 850) for the installation of a *Crosstalk Color Compensation* file
- *LightCycler* Capillaries (20 µl)
- *LightCycler* Cooling Block
- *LightCycler* Instrument
- *LightCycler* Capping Tool

4. General Precautions

The user should always pay attention to the following:

- Use sterile pipette tips with filters.
- Store and extract positive material (specimens, controls and amplicons) separately from all other reagents and add it to the reaction mix in a spatially separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Work quickly on ice or in the *LightCycler* Cooling Block.

5. Pathogen Information

Herpes simplex virus (HSV) is found in lesion fluids, saliva 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.

6. Principle of Real-Time PCR

Pathogen diagnosis 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 which 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 (Mackay, 2004).

7. Product Description

The *artus* HSV-1/2 LC PCR Kit constitutes a ready-to-use system for the detection and differentiation of herpes simplex virus-1 and -2 DNA using polymerase chain reaction (PCR) followed by melting curve analysis in the *LightCycler* Instrument. The *HSV LC Master* contains reagents and enzymes for the specific amplification of a 148 bp region of the herpes simplex virus genome, and for the direct detection of the specific amplicon in fluorimeter channel F2 of the *LightCycler* Instrument. In addition, the *artus* HSV-1/2 LC Kit contains a second heterologous amplification system to identify possible PCR inhibition. This is detected as an *Internal Control (IC)* in fluorimeter channel F3. The detection limit of the analytical HSV PCR (see 11.1 Analytical Sensitivity) is not reduced. In order to distinguish one subtype from the other, the system utilizes the specific melting temperature of the probes. During the melting curve step, a signal is detected in fluorimeter channel F2 for HSV-1 at 69°C and for HSV-2 at 66°C. Depending on the various extraction conditions and as a result of their respective buffering conditions, these values can deviate by 1 – 2°C. However, this deviation will be equal for both subtypes. External positive controls (*HSV1 LC/RG/TM QS 1 – 4* & *HSV2 LC/RG/TM QS 1 – 4*) are supplied which allow the determination of the pathogen load. For further information, please refer to section 8.3 Quantitation.

Attention: The temperature profile for the detection of HSV-1 and HSV-2 using the *artus* HSV-1/2 LC PCR Kit corresponds to the profiles of the *artus* EBV LC PCR Kit, the *artus* VZV LC PCR Kit and the *artus* CMV LC PCR Kit. Therefore, the PCR assays of these *artus* systems can be carried out and analysed in one single run. Please note the recommendations on PCR analysis given in chapters 8.3 Quantitation and 9. Data Analysis.

8. Protocol

8.1 DNA Isolation

Various manufacturers offer DNA isolation kits. Sample amounts for the DNA isolation procedure depend on the protocol used. Please carry out the DNA isolation according to the manufacturer's instructions. The following isolation kits are recommended:

Sample Material	Nucleic Acid Isolation Kit	Catalogue Number	Manufacturer	Carrier RNA
Serum, plasma, CSF, swabs	QIAamp® UltraSens® Virus Kit (50)	53 704	QIAGEN	included
	QIAamp DNA Mini Kit (50)	51 304	QIAGEN	not included
CSF	EZ1® DSP Virus Kit (48)*	62 724	QIAGEN	included

*To be used in combination with the BioRobot® EZ1 DSP Workstation (Cat. No. 9001360) and the EZ1 DSP Virus Card (Cat. No. 9017707).

Important note for the use of the QIAamp UltraSens Virus Kit and the QIAamp DNA Mini Kit:

- The use of carrier RNA is critical for the extraction efficiency and, consequently, for DNA/RNA yield. If the selected isolation kit does not contain carrier RNA, please note that the addition of carrier (RNA-Homopolymer Poly(A), Amersham Biosciences, Cat. No. 27-4110-01) is strongly recommended for the extraction of nucleic acids from cell free body fluids and material low in DNA/RNA content (e.g. CSF). Please proceed as follows in these cases:

  a) Resuspend the lyophilised carrier RNA using the elution buffer (do not use lysis buffer) of the extraction kit (e.g. AE buffer of the QIAamp DNA Mini Kit) and prepare a dilution with a concentration of 1 µg/µl. Divide this carrier RNA solution in a number of aliquots adequate to your needs and store them at –20°C. Avoid repeated thawing (> 2 x) of a carrier RNA aliquot.

  b) Use 1 µg carrier RNA per 100 µl lysis buffer. For instance, if the extraction protocol suggests 200 µl lysis buffer, please add 2 µl carrier RNA (1 µg/µl) directly into the lysis buffer. Before beginning of each extraction, a mixture of lysis buffer and carrier RNA (and *Internal Control*, where applicable, see 8.2 *Internal Control*) should be prepared freshly according to the following pipetting scheme:

Number of samples	1	12
Lysis buffer	e.g. 200 µl	e.g. 2,400 µl
Carrier RNA (1 µg/µl)	2 µl	24 µl
Total Volume	202 µl	2,424 µl
Volume per extraction	200 µl each	200 µl

c) Please use the freshly prepared mixture of lysis buffer and carrier RNA instantly for extraction. Storage of the mixture is not possible.

- The use of carrier RNA is critical for the extraction efficiency and, consequently, for DNA/RNA yield. To increase the stability of the carrier RNA provided with the QIAamp UltraSens Virus Kit, we recommend the following procedure deviant from the user manual of the extraction kit:

a) Resuspend the lyophilised carrier RNA prior to first use of the extraction kit in 310 µl of the elution buffer provided with the kit (final concentration 1 µg/µl, do not use lysis buffer). Portion this carrier RNA solution into a number of aliquots adequate to your needs and store them at –20°C. Avoid repeated thawing (> 2 x) of a carrier RNA aliquot.

b) Before the beginning of each extraction, a mixture of lysis buffer and carrier RNA (and *Internal Control*, where applicable, see 8.2 *Internal Control*) should be prepared freshly according to the following pipetting scheme:

Number of samples	1	12
Lysis buffer AC	800 µl	9,600 µl
Carrier RNA (1 µg/µl)	5.6 µl	67.2 µl
Total Volume	805.6 µl	9,667.2 µl
Volume per extraction	800 µl each	800 µl

c) Please use the freshly prepared mixture of lysis buffer and carrier RNA instantly for extraction. Storage of the mixture is not possible.

- It is recommended to elute the DNA in 50 µl elution buffer to get the highest sensitivity of the *artus* HSV-1/2 LC PCR Kit.
- The **QIAamp UltraSens Virus Kit** allows a sample concentration. If you use sample material other than serum or plasma, please add at least 50 % (v/v) of negative human plasma to the sample.

- When using isolation protocols with **ethanol**-containing washing buffers, please carry out an additional centrifugation step (three minutes, 13,000 rpm) before the elution to remove any remaining ethanol. This prevents possible inhibition of PCR.
- The *artus* HSV-1/2 LC PCR Kit should not be used with **phenol**-based isolation methods.

Important note for the use of the EZ1 DSP Virus Kit:

- The use of **carrier RNA** is critical for the extraction efficiency and, consequently, for DNA/RNA yield. Please add the appropriate amount of carrier RNA to each extraction following the instructions in the *EZ1 DSP Virus Kit Handbook*.

Important: The *Internal Control* of the *artus* HSV-1/2 LC PCR Kit can be used directly in the isolation procedure (see 8.2 *Internal Control*).

8.2 *Internal Control*

An *Internal Control* (*HSV LC IC*) is supplied. This allows the user **both to control the DNA isolation procedure and to check for possible PCR inhibition** (see Fig. 1). Using the **EZ1 DSP Virus Kit** for extraction, the *Internal Control* has to be added following the instructions in the *EZ1 DSP Virus Kit Handbook*. Using the **QIAamp UltraSens Virus Kit** or the **QIAamp DNA Mini Kit**, add the *Internal Control* to the isolation at a ratio of 0.1 µl per 1 µl elution volume. For example, using the QIAamp DNA Mini Kit (QIAGEN) the DNA is eluted in 50 µl AE buffer. Hence, 5 µl of the *Internal Control* should be added initially. The quantity of *Internal Control* used depends **only** on the elution volume. The *Internal Control* and carrier RNA (see 8.1 DNA Isolation) 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 instantly (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). Please 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** (see Fig. 2). For this application, add 0.5 µl of the *Internal Control* per reaction directly to 15 µl *HSV LC Master*. For each PCR reaction use 15 µl of the Master Mix produced as described above* and add 5 µl of the

* 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.

purified sample. If you are preparing a PCR run for several samples please increase the volume of the *HSV LC Master* and the *Internal Control* according to the number of samples (see 8.4 Preparing the PCR).

The *artus* HSV-1/2 LC PCR Kits and the *artus* VZV LC PCR Kits contain an identical *Internal Control* (IC). The *artus* EBV LC PCR Kits and the *artus* CMV LC PCR Kits also contain an identical *Internal Control*.

8.3 Quantitation

The enclosed *Quantitation Standards* (*HSV1 LC/RG/TM QS 1 – 4* & *HSV2 LC/RG/TM QS 1 – 4*) are treated as previously purified samples and the same volume is used (5 µl). To generate a standard curve on the *LightCycler* Instrument, all four *Quantitation Standards* of HSV-1 as well as HSV-2 should be used and defined in the *Sample Loading Screen* as standards with the specified concentrations (see *LightCycler Operator's Manual*, Version 3.5, Chapter B, 2.4. Sample Data Entry). The standard curve generated as above can also be used for subsequent runs, provided that at least one standard of **one** given concentration is used in the current run. For this purpose, the previously generated standard curve needs to be imported (see *LightCycler Operator's Manual*, Version 3.5, Chapter B, 4.2.5. Quantitation with an External Standard Curve). However, this quantitation method may lead to deviations in the results due to variability between different PCR runs.

If you integrated more than one Herpes *artus* system in the PCR run, please analyse these different systems with the corresponding *Quantitation Standards* separately.

Attention: The *Quantitation Standards* are defined as copies/µl. The following equation has to be applied to convert the values determined using the standard curve into copies/ml of sample material:

$$\text{Result (copies/ml)} = \frac{\text{Result (copies/µl)} \times \text{Elution Volume (µl)}}{\text{Sample Volume (ml)}}$$

Please note that as a matter of principle the initial sample volume should be entered in the equation above. This has to be considered when the sample volume has been changed prior to the nucleic acid extraction (e.g. narrowing the volume by centrifugation or increase of volume by replenishment to the volume required for the isolation).

Important: A guideline for the quantitative analysis of *artus* systems on the *LightCycler* Instrument is provided at **www.qiagen.com/Products/ByLabFocus/MDX** (**Technical Note for quantitation on the *LightCycler* 1.1/1.2/1.5 or *LightCycler* 2.0 Instrument**).

8.4 Preparing the PCR

Make sure that the Cooling Block as well as the capillary adapters (accessories of the *LightCycler* Instrument) are pre-cooled to +4°C. Place the desired number of *LightCycler* capillaries into the adapters of the Cooling Block. Please make sure that at least one *Quantitation Standard* (*HSV1 LC/RG/TM QS 1 – 4* & *HSV2 LC/RG/TM QS 1 – 4*) as well as one negative control (*Water, PCR grade*) are included per PCR run. To generate a standard curve, use all supplied *Quantitation Standards* (*HSV1 LC/RG/TM QS 1 – 4* & *HSV2 LC/RG/TM QS 1 – 4*) for each PCR run. Before each use, all reagents need to be thawed completely, mixed (by repeated up and down pipetting or by quick vortexing) and centrifuged briefly.

If you want to use the *Internal Control* **to monitor the DNA isolation procedure and to check for possible PCR inhibition,** it has already been added to the isolation (see 8.2 *Internal Control*). In this case, please use the following pipetting scheme (for a schematic overview see Fig. 1):

	Number of samples	**1**	**12**
1. Preparation of Master Mix	*HSV LC Master*	15 µl	180 µl
	HSV LC IC	0 µl	0 µl
	Total Volume	15 µl	180 µl
2. Preparation of PCR assay	Master Mix	15 µl	15 µl each
	Sample	5 µl	5 µl each
	Total Volume	20 µl	20 µl each

If you want to use the *Internal Control* **exclusively to check for PCR inhibition**, it must be added directly to the *HSV LC Master*. In this case, please use the following pipetting scheme (for a schematic overview see Fig. 2):

	Number of samples	**1**	**12**
1. Preparation of Master Mix	*HSV LC Master*	15 µl	180 µl
	HSV LC IC	0.5 µl	6 µl
	Total Volume	15.5 µl*	186 µl*
2. Preparation of PCR assay	Master Mix	15 µl*	15 µl each*
	Sample	5 µl	5 µl each
	Total Volume	20 µl	20 µl each

* 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.

Pipette 15 µl of the Master Mix into the plastic reservoir of each capillary. Then add 5 µl of the eluted sample DNA. Correspondingly, 5 µl of at least one *Quantitation Standard* of each of the standard series (*HSV1 LC/RG/TM QS 1– 4* & *HSV2 LC/RG/TM QS 1 – 4*) must be used as a positive control and 5 µl of water (*Water, PCR grade*) as a negative control. 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 ten seconds at a maximum of 400 x *g* (2,000 rpm).

Addition of the *Internal Control* to the Purification Procedure

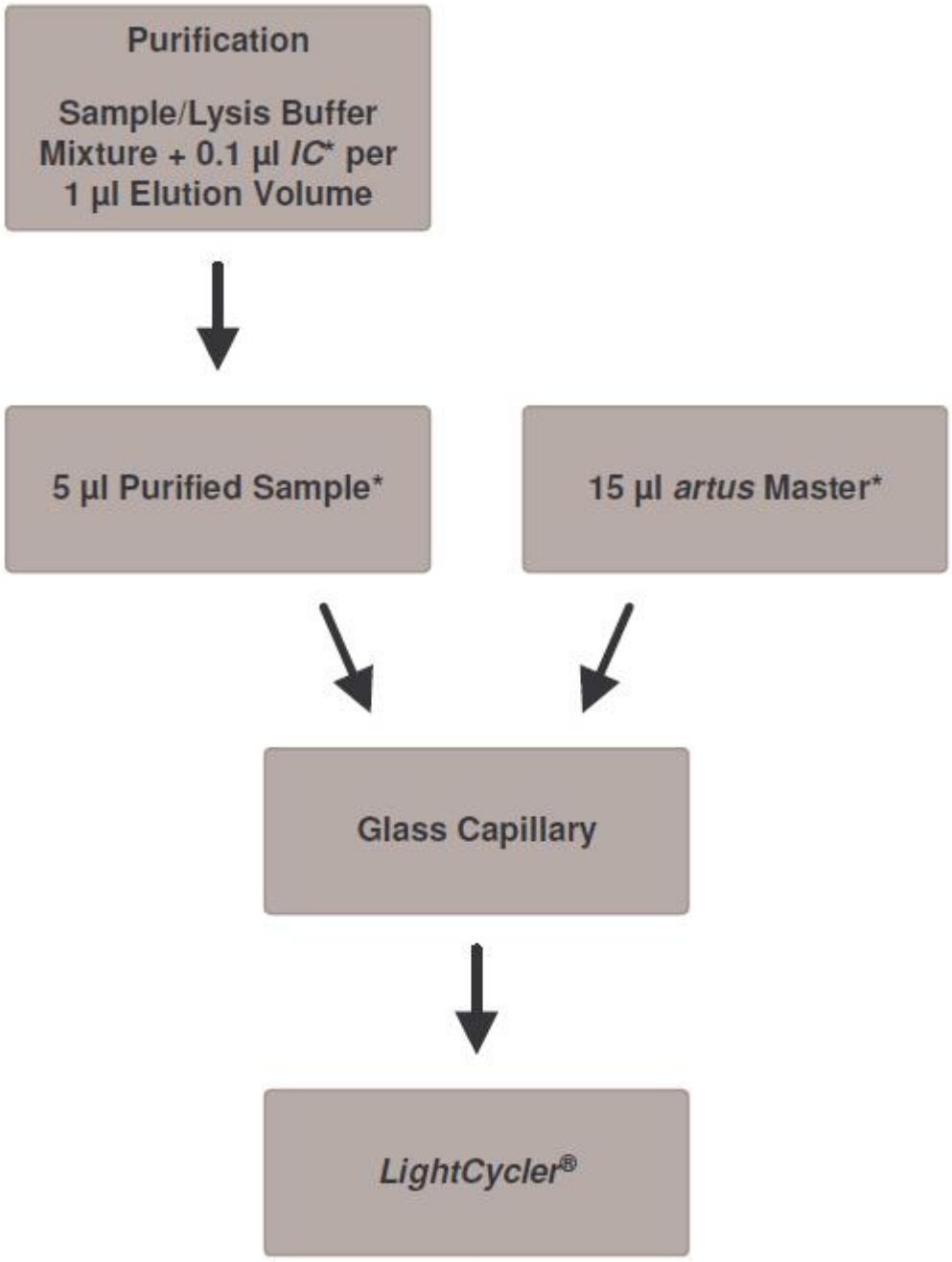

Fig. 1: Schematic workflow for the control of both the purification procedure and PCR inhibition.

* Please make sure that the solutions are thawed completely, mixed well and centrifuged briefly.

Addition of the *Internal Control* into the *artus* Master

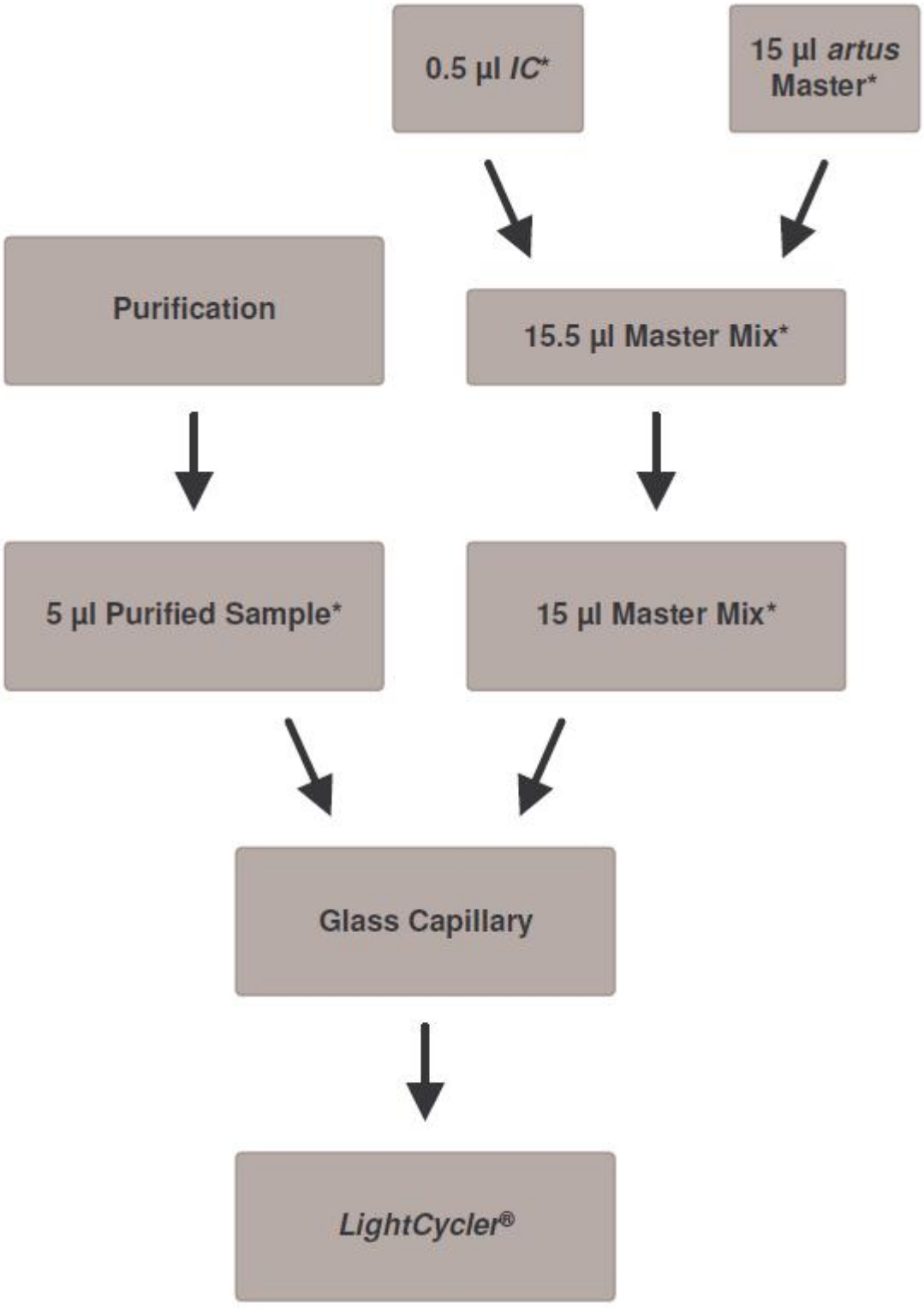

Fig. 2: Schematic workflow for the control of PCR inhibition.

* Please make sure that the solutions are thawed completely, mixed well and centrifuged briefly.

8.5 Programming the *LightCycler* Instrument

For the detection of herpes simplex virus DNA, create a temperature profile on your *LightCycler* Instrument according to the following five steps (see Fig. 3 – 7).

A. Initial Activation of the Hot Start Enzyme Fig. 3

B. Touch Down Step Fig. 4

C. Amplification of the DNA Fig. 5

D. Melting Curve Fig. 6

E. Cooling Fig. 7

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. Please find further information on programming the *LightCycler* Instrument in the *LightCycler Operator's Manual*.

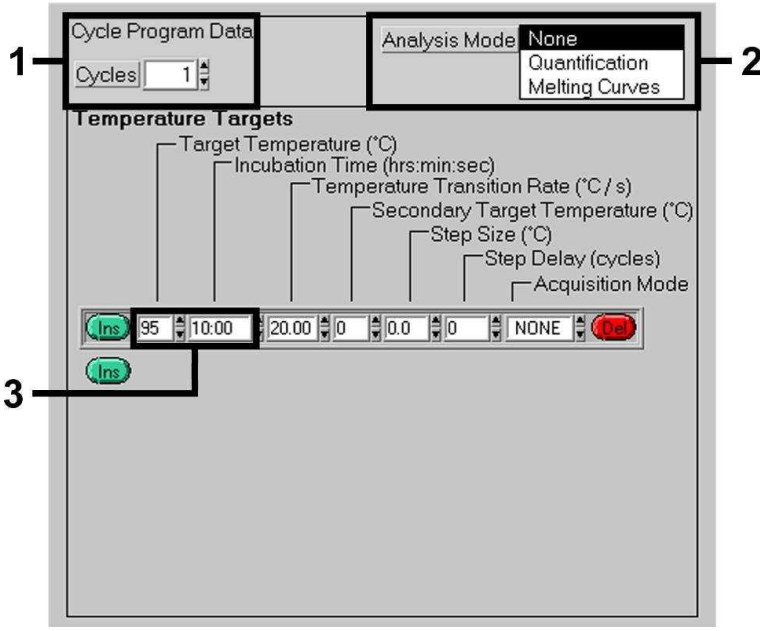

Fig. 3: Initial Activation of the Hot Start Enzyme.

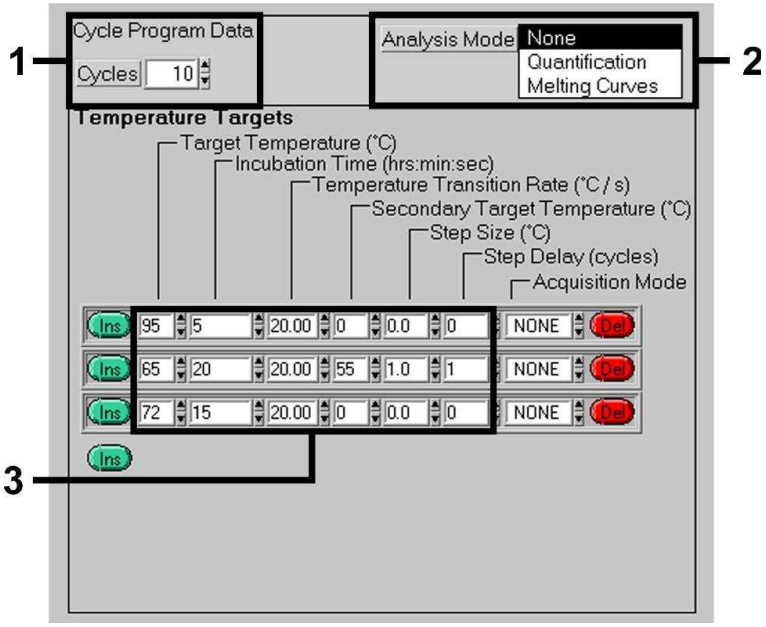

Fig. 4: Touch Down Step.

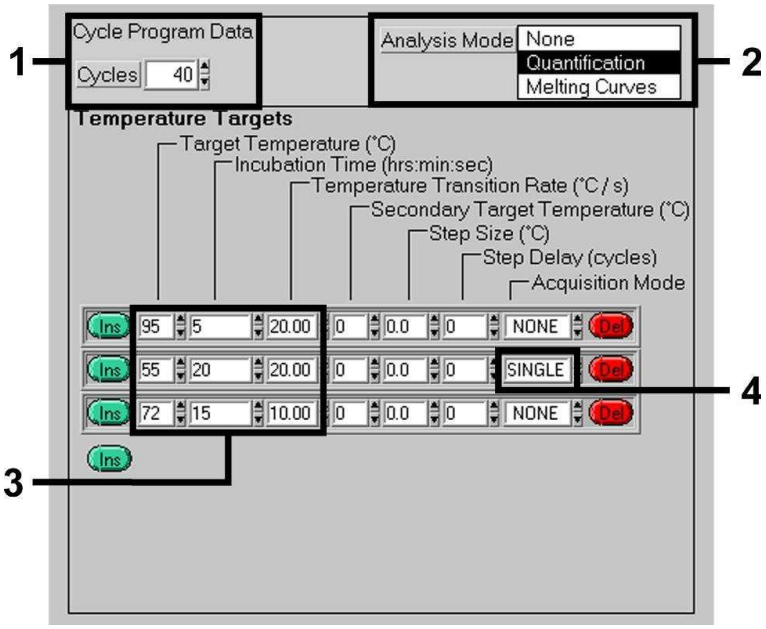

Fig. 5: Amplification of the DNA.

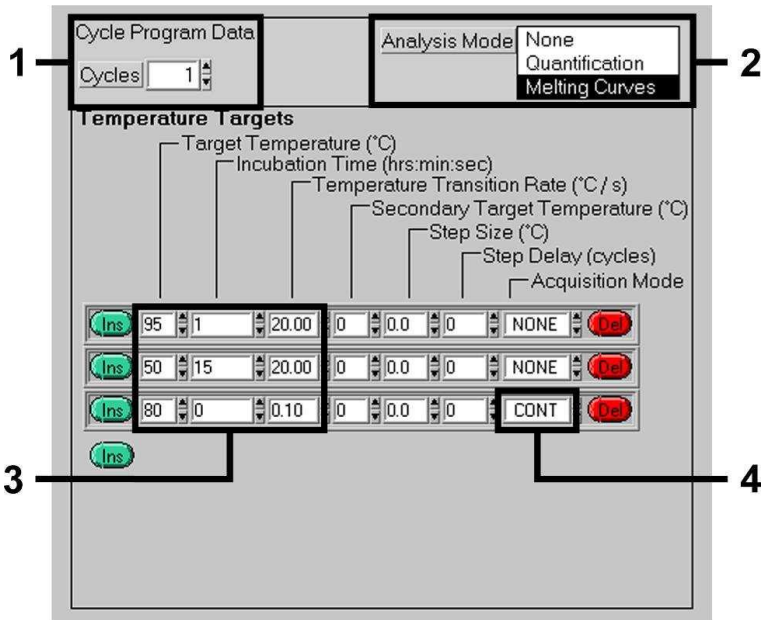

Fig. 6: Melting Curve.

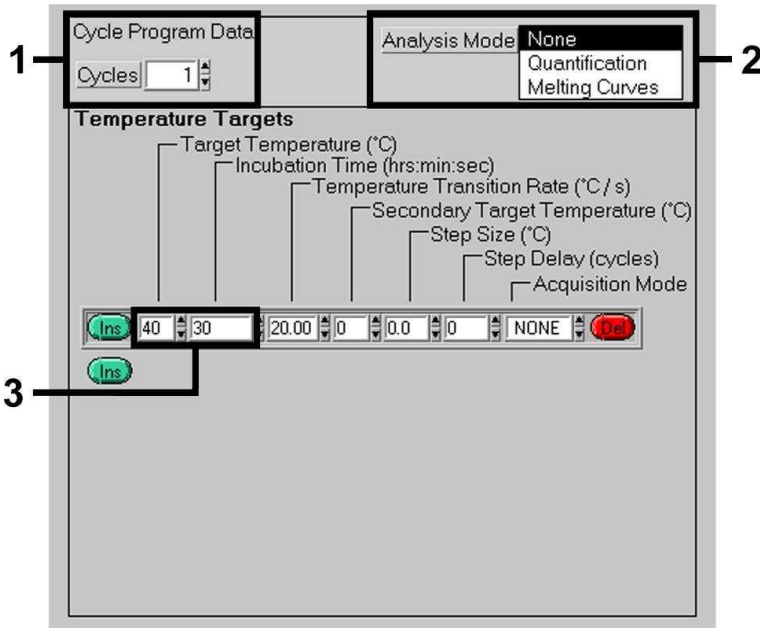

Fig. 7: Cooling.

9. Data Analysis

In multicolour 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 activating the *Choose CCC File* or the *Select CC Data* button. If no *Color Compensation File* is installed, generate the file according to the instructions in the *LightCycler Operator's Manual*. After the *Color Compensation File* has been activated, separate signals appear in fluorimeter channels F1, F2 and F3. For analysis of the PCR results gained with the *artus* HSV-1/2 LC PCR Kit please select fluorescence display options F2/Back-F1 for

the analytical HSV PCR and F3/Back-F1 for the *Internal Control* PCR, respectively. For the analysis of quantitative runs, please follow the instructions given in 8.3 Quantitation and in the **Technical Note for quantitation on the *LightCycler* 1.1/1.2/1.5 or *LightCycler* 2.0 Instrument** at **www.qiagen.com/Products/ByLabFocus/MDX**.

If you integrated more than one Herpes *artus* system in the PCR run, please analyse these different systems with the corresponding *Quantitation Standards* separately. This also applies to the analysis of the two HSV subtypes. Thus, for the quantitation of HSV-1 samples refer to the standard curve generated with the HSV-1 standards (*HSV1 LC/RG/TM QS 1 – 4*). Proceed accordingly for HSV-2 using the standard curve based on the analysis of the HSV-2 standards (*HSV2 LC/RG/TM QS 1 – 4*).

The following results are possible:

1. A signal is detected in fluorimeter channel F2/Back-F1.

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

   In this case, the detection of a signal in the F3/Back-F1 channel is dispensable, since high initial concentrations of HSV DNA (positive signal in the F2/Back-F1 channel) can lead to a reduced or absent fluorescence signal of the *Internal Control* in the F3/Back-F1 channel (competition).

   Differentiation can be made between the HSV-1 and HSV-2 amplicons on the basis of melting points (channel F2/Back-F1, programme *melting curve*); that for HSV-1, should be 69°C and for HSV-2, 66°C. Depending on the various extraction conditions and as a result of their respective buffering conditions, these values can deviate by 1 – 2°C. However, this deviation will be equal for both subtypes.

2. In fluorimeter channel F2/Back-F1 no signal is detected. At the same time, a signal from the *Internal Control* appears in the F3/Back-F1 channel.

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

   In the case of a negative HSV PCR the detected signal of the *Internal Control* rules out the possibility of PCR inhibition.

3. No signal is detected in the F2/Back-F1 or in the F3/Back-F1 channel.

   No diagnosis can be concluded.

   Information regarding error sources and their solution can be found in 10. Troubleshooting.

Examples of positive and negative PCR reactions as well as the melting curves for differentiation are given in Fig. 8 to Fig. 12.

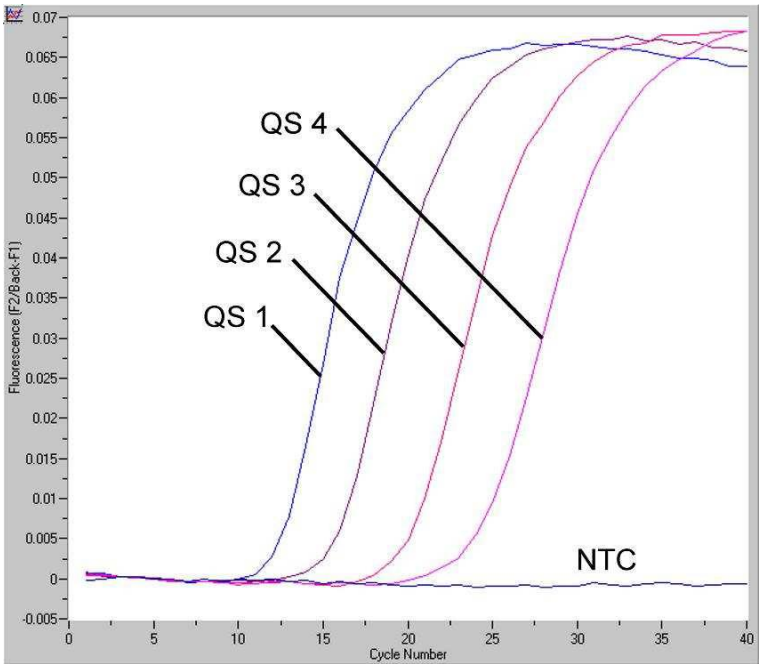

Fig. 8: Detection of the *Quantitation Standards* (***HSV1*** *LC/RG/TM QS 1 – 4*) in fluorimeter channel F2/Back-F1. NTC: nontemplate control (negative control).

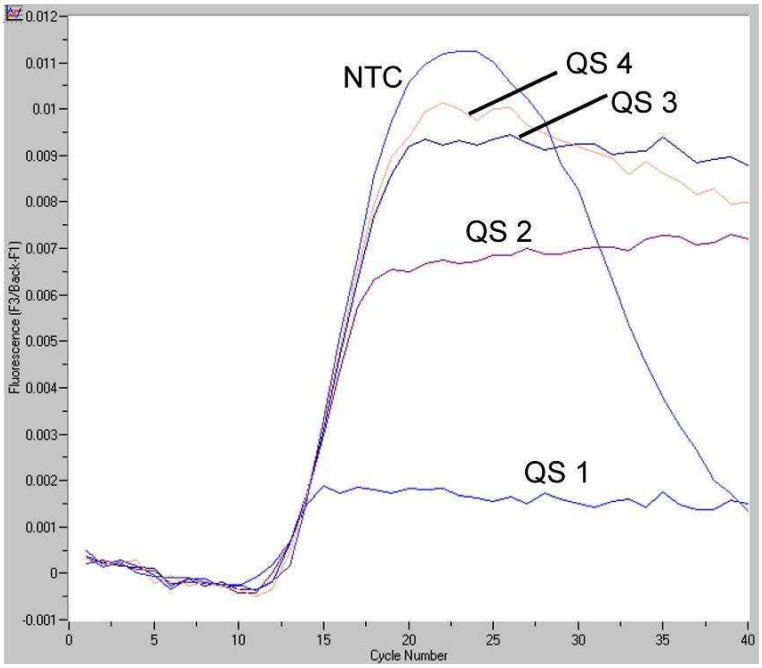

Fig. 9: Detection of the *Internal Control* (IC) in fluorimeter channel F3/Back-F1 with simultaneous amplification of *Quantitation Standards* (***HSV1*** *LC/RG/TM QS 1 – 4*). NTC: non-template control (negative control).

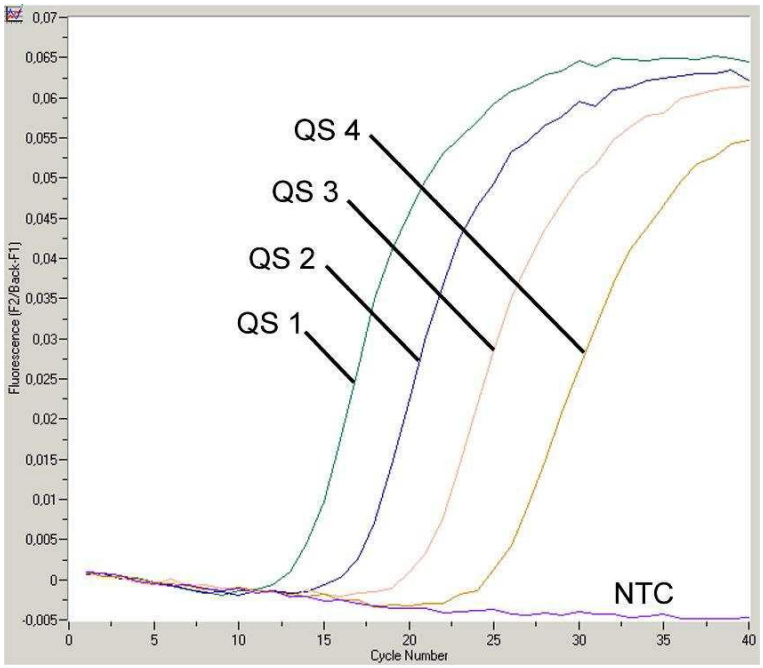

Fig. 10: Detection of the *Quantitation Standards* (***HSV2*** *LC/RG/TM QS 1 – 4*) in fluorimeter channel F2/Back-F1. NTC: nontemplate control (negative control).

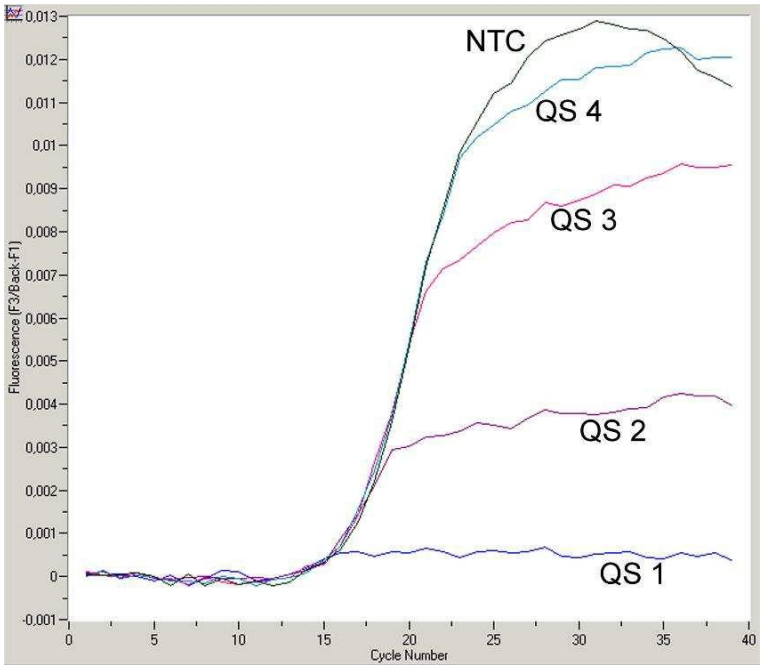

Fig. 11: Detection of the *Internal Control* (IC) in fluorimeter channel F3/Back-F1 with simultaneous amplification of *Quantitation Standards* (**HSV2** LC/RG/

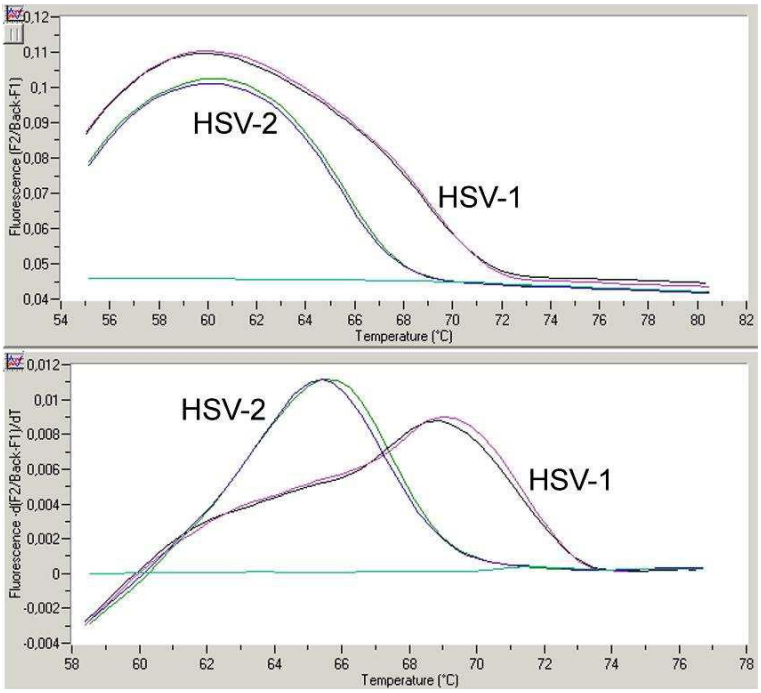

Fig. 12: Demonstration of the differentiation between HSV-1 and HSV-2 in fluorimeter channel **F2/Back-F1** (Programme *Melting Curve*).

10. Troubleshooting

No signal with positive controls (*HSV1 LC/RG/TM QS 1 – 4* & *HSV2 LC/RG/TM QS 1 – 4*) in fluorimeter channel F2/Back-F1:

- The selected fluorimeter channel for PCR data analysis does not comply with the protocol.

  ➔ For data analysis select the fluorimeter channel F2/Back-F1 for the analytical HSV PCR and the fluorimeter channel F3/Back-F1 for the *Internal Control* PCR.

- Incorrect programming of the temperature profile of the *LightCycler* Instrument.

  ➔ Compare the temperature profile with the protocol (see 8.5 Programming of the *LightCycler* Instrument).

- Incorrect configuration of the PCR reaction.

  ➔ Check your work steps by means of the pipetting scheme (see 8.4 Preparing the PCR) and repeat the PCR, if necessary.

- The storage conditions for one or more kit components did not comply with the instructions given in 2. Storage or the *artus* HSV-1/2 LC PCR Kit had expired.

  ➔ Please 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* in fluorimeter channel F3/Back-F1 and simultaneous absence of a signal in channel F2/Back-F1:

- The PCR conditions do not comply with the protocol.
  - ➔ Check the PCR conditions (see above) and repeat the PCR with corrected settings, if necessary.
- The PCR was inhibited.
  - ➔ Make sure that you use a recommended isolation method (see 8.1 DNA Isolation) and stick closely to the manufacturer's instructions.
  - ➔ Make sure that during the DNA isolation the recommended additional centrifugation step has been carried out before the elution in order to remove any residual ethanol (see 8.1 DNA Isolation).
- DNA was lost during extraction.
  - ➔ If the *Internal Control* had been 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 a recommended isolation method (see 8.1 DNA Isolation) and stick closely to the manufacturer's instructions.
- The storage conditions for one or more kit components did not comply with the instructions given in 2. Storage or the *artus* HSV-1/2 LC PCR Kit had expired.
  - ➔ Please 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 fluorimeter channel F2/Back-F1 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.
  - ➔ Strictly pipette the positive controls at last.
  - ➔ Make sure that work space and instruments are decontaminated at regular intervals.
- A 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.

If you have any further questions or if you encounter problems, please contact our Technical Service.

11. Specifications

11.1 Analytical Sensitivity

In order to determine the analytical sensitivity of the *artus* HSV-1/2 LC PCR Kit, a standard dilution series has been set up from 31.6 to nominal 0.01 HSV-1 and HSV-2 copy equivalents*/µl and analysed with the *artus* HSV-1/2 LC PCR Kit. Testing was carried out on three different days on eight replicates. The results were determined by a probit analysis. A graphical illustration of the probit analyses is shown in Fig. 13 and 14. The analytical detection limit of the *artus* HSV-1/2 LC PCR Kit is for HSV-1 and HSV-2 consistently 1 copy/µl (p = 0.05). This means that there is a 95 % probability that 1 copy/µl will be detected.

Probit analysis: Herpes simplex virus 1 (*LightCycler*)

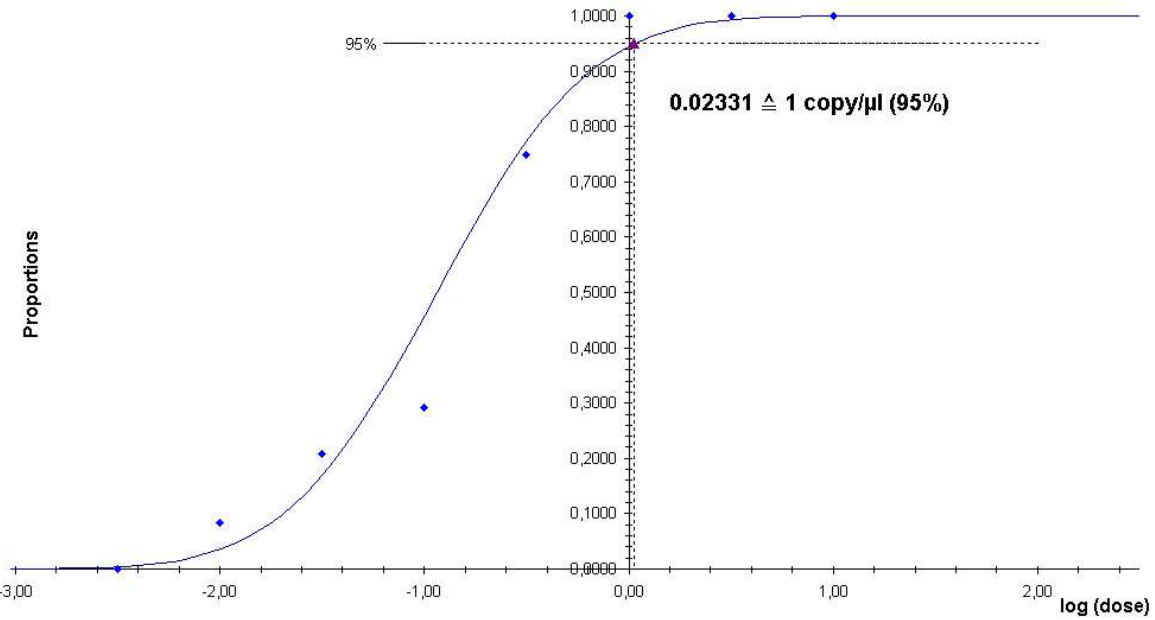

Fig. 13: Analytical sensitivity of the *artus* HSV-1/2 LC PCR Kit (**HSV-1**).

* The standard is a cloned PCR product, the concentration of which has been determined by absorption and fluorescence spectroscopy.

Probit analysis: Herpes simplex virus 2 (*LightCycler*)

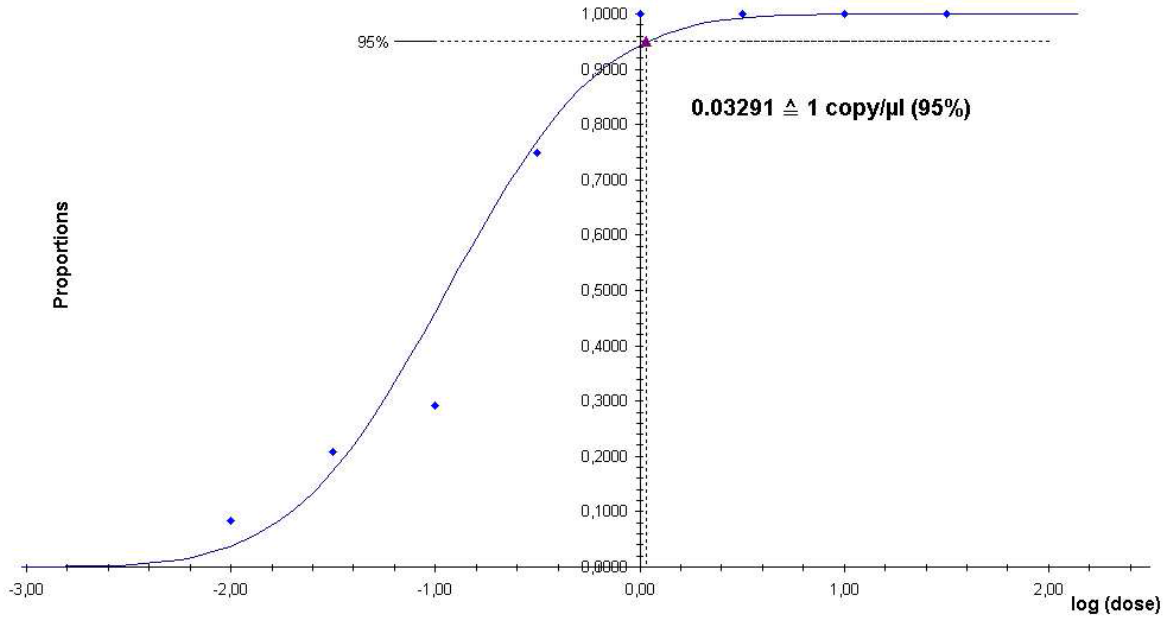

Fig. 14: Analytical sensitivity of the *artus* HSV-1/2 LC PCR Kit (**HSV-2**).

11.2 Specificity

The specificity of the *artus* HSV-1/2 LC PCR Kit is first and foremost ensured by the selection of the primers and probes, as well as the selection of stringent reaction conditions. The primers and probes were checked for possible homologies to all in gene banks published sequences by sequence comparison analysis. The detectability of all relevant strains has thus been ensured.

Moreover, the specificity was validated with 30 different HSV negative cerebrospinal fluid samples. These did not generate any signals with the HSV specific primers and probes, which are included in the *HSV LC Master*.

To determine the specificity of the *artus* HSV-1/2 LC PCR Kit the control group listed in the following table (see Table 1) has been tested for cross-reactivity. None of the tested pathogens has been reactive.

Table 1. Testing the specificity of the kit with potentially cross-reactive pathogens

Control Group	HSV-1/2 (F2/Back-F1)	*Internal Control* (F3/Back-F1)
Human herpesvirus 3 (Varicella-zoster virus)	–	+
Human herpesvirus 4 (Epstein-Barr virus)	–	+
Human herpesvirus 5 (Cytomegalovirus)	–	+
Human herpesvirus 6 A	–	+
Human herpesvirus 6 B	–	+
Human herpesvirus 7	–	+
Human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus)	–	+

11.3 Precision

The precision data of the *artus* HSV-1/2 LC PCR Kit allow the determination of the total variance of the assay. The total variance consists of the **intra-assay variability** (variability of multiple results of samples of the same concentration within one experiment), the **inter-assay variability** (variability of multiple results of the assay generated on different instruments of the same type by different operators within one laboratory) and the **inter-batch variability** (variability of multiple results of the assay using various batches). The data obtained were used to determine the standard deviation, the variance and the coefficient of variation for the pathogen specific and the *Internal Control* PCR.

Precision data of the *artus* HSV-1/2 LC PCR Kit have been collected using the *Quantitation Standard* of the lowest concentration (QS 4; 10 copies/µl). Testing was performed with eight replicates. The precision data were calculated on basis of the Ct values of the amplification curves (Ct: threshold cycle, see Table 2/Table 4). In addition, precision data for quantitative results in copies/µl were determined using the corresponding Ct values (see Table 3/Table 5). Based on these results, the overall statistical spread of any given sample with the mentioned concentration is 1.67 % (Ct; HSV-1) and 1.95 % (Ct; HSV-2) or 20.66 % (conc., HSV-1) and 22.42 % (conc., HSV-2), for the detection of the *Internal Control* 1.23 % (Ct; HSV-1) and 1.04 % (Ct; HSV-2). These values are based on the totality of all single values of the determined variabilities.

Table 2. Precision data for HSV-1 on basis of the Ct values

	Standard Deviation	Variance	Coefficient of Variation [%]
Intra-assay variability: *HSV1 LC/RG/TM QS 4*	0.27	0.07	1.13
Intra-assay variability: *Internal Control*	0.03	0.00	0.23
Inter-assay variability: *HSV1 LC/RG/TM QS 4*	0.39	0.15	1.66
Inter-assay variability: *Internal Control*	0.12	0.01	0.99
Inter-batch variability: *HSV1 LC/RG/TM QS 4*	0.41	0.17	1.72
Inter-batch variability: *Internal Control*	0.17	0.03	1.40
Total variance: *HSV1 LC/RG/TM QS 4*	0.39	0.15	1.67
Total variance: *Internal Control*	0.15	0.02	1.23

Table 3. Precision data for HSV-1 on basis of the quantitative results (in copies/µl)

	Standard Deviation	Variance	Coefficient of Variation [%]
Intra-assay variability: *HSV1 LC/RG/TM QS 4*	1.76	3.08	17.34
Inter-assay variability: *HSV1 LC/RG/TM QS 4*	2.02	4.08	19.82
Inter-batch variability: *HSV1 LC/RG/TM QS 4*	2.37	5.64	23.10
Total variance: *HSV1 LC/RG/TM QS 4*	2.11	4.46	20.66

Table 4: Precision data for HSV-2 on basis of the Ct values

Herpes simplex virus 2	Standard Deviation	Variance	Coefficient of Variation [%]
Intra-assay variability: *HSV2 LC/RG/TM QS 4*	0.22	0.05	0.90
Intra-assay variability: *Internal Control*	0.04	0.00	0.33
Inter-assay variability: *HSV2 LC/RG/TM QS 4*	0.62	0.38	2.51
Inter-assay variability: *Internal Control*	0.12	0.01	0.98
Inter-batch variability: *HSV2 LC/RG/TM QS 4*	0.38	0.14	1.52
Inter-batch variability: *Internal Control*	0.14	0.02	1.12
Total variance: *HSV2 LC/RG/TM QS 4*	0.48	0.23	1.95
Total variance: *Internal Control*	0.13	0.02	1.04

Table 5. Precision data for HSV-2 on basis of the quantitative results (in copies/µl)

Herpes simplex virus 2	Standard Deviation	Variance	Coefficient of Variation [%]
Intra-assay variability: *HSV2 LC/RG/TM QS 4*	1.39	1.94	13.82
Inter-assay variability: *HSV2 LC/RG/TM QS 4*	2.86	8.20	27.46
Inter-batch variability: *HSV2 LC/RG/TM QS 4*	1.96	3.85	19.27
Total variance: *HSV2 LC/RG/TM QS 4*	2.30	5.31	22.42

11.4 Robustness

The verification of the robustness allows the determination of the total failure rate of the *artus* HSV-1/2 LC PCR Kit. 30 HSV negative samples of cerebrospinal fluid were spiked with 3 copies/µl of elution volume of HSV-1 control DNA (threefold concentration of the detection limit). After extraction using the QIAamp DNA Mini Kit (QIAGEN; see 8.1 DNA Isolation) these samples were analysed with the *artus* HSV-1/2 LC PCR Kit. The analysis of HSV-2 has been carried out similarly (30 cerebrospinal fluid samples, 3 copies/µl HSV-2 control DNA). For all HSV-1 and HSV-2 samples the failure rate was 0 %. In addition, the robustness of the *Internal Control* was assessed by purification and analysis of 30 HSV negative cerebrospinal fluid samples. The total failure rate was 0 %. Inhibitions were not observed. Thus, the robustness of the *artus* HSV-1/2 LC PCR Kit is ≥ 99 %.

11.5 Reproducibility

Reproducibility data permit a regular performance assessment of the *artus* HSV-1/2 LC PCR Kit as well as an efficiency comparison with other products. These data are obtained by the participation in established proficiency programmes.

11.6 Diagnostic Evaluation

Currently, the *artus* HSV-1/2 LC PCR Kit is undergoing a series of evaluation studies.

12. Product Use Limitations

- All reagents may exclusively be used in in vitro diagnostics.
- The product is to be used by personnel specially instructed and trained in the in vitro diagnostics procedures only.
- 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.

13. Safety information

For safety information of the *artus* HSV-1/2 LC PCR Kit, please consult the appropriate safety data sheet (SDS). The SDSs are available online in convenient and compact PDF format at **www.qiagen.com/safety**.

14. Quality control

In accordance with QIAGEN's ISO 9001 and ISO 13485-certified Quality Management System, each lot of *artus* HSV-1/2 LC PCR Kit has been tested against predetermined specifications to ensure consistent product quality.

15. References

(1) Mackay IM. Real-time PCR in the microbiology laboratory. Clin. Microbiol. Infect. 2004; 10 (3): 190 – 212.

(2) Whiley DM, Syrmis MW, Mackay IM, Sloots TP. Preliminary comparison of three *LightCycler* PCR assays for the detection of Herpes Simplex virus in swab specimens. Eur J Clin Microbiol Infect Dis. 2003; 22: 764 – 767.

16. Explanation of Symbols


Use by

Batch code


Manufacturer

Catalogue number

Material number

Handbook

In vitro diagnostic medical device

Ethanol

Global Trade Item Number

<N>
Contains sufficient for <N> tests


Temperature limitation

QS *Quantitation Standard*

IC *Internal Control*

artus HSV-1/2 LC PCR Kit

Trademarks and Disclaimers

QIAGEN®, QIAamp®, *artus*®, BioRobot®, EZ1®, UltraSens® (QIAGEN Group); *Light Cycler*® (Roche Diagnostics).

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The *artus* HSV-1/2 LC PCR Kit, the BioRobot EZ1 DSP Workstation, and the EZ1 DSP Virus Kit and Card are CE-marked diagnostic devices according to the European In Vitro Diagnostic Directive 98/78/EC. Not available in all countries.

The QIAamp Kits are intended for general laboratory use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Purchase of *artus* PCR Kits is accompanied by a limited license to use them in the polymerase chain reaction (PCR) process for human and veterinary in vitro diagnostics in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Applied Biosystems or as purchased, i.e. an authorized thermal cycler. The PCR process is covered by the foreign counterparts of U.S. Patents Nos. 5,219,727 and 5,322,770 and 5,210,015 and 5,176,995 and 6,040,166 and 6,197,563 and 5,994,056 and 6,171,785 and 5,487,972 and 5,804,375 and 5,407,800 and 5,310,652 and 5,994,056 owned by F. Hoffmann-La Roche Ltd.

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