

artus[®] CMV LC PCR Kit Handbook

 24 (catalog no. 4503063)

 96 (catalog no. 4503065)

Quantitative in vitro Diagnostics

For use with the *LightCycler*[®] 1.1/1.2/1.5 and *LightCycler* 2.0
Instrument

December 2014 — Version 1



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artus CMV LC PCR Kit

For use with the *LightCycler 1.1/1.2/1.5* or *LightCycler 2.0* Instrument for the quantitative detection of CMV DNA from EDTA plasma.

1. Contents

	Labeling and contents	Art. No. 4503063 24 reactions	Art. No. 4503065 96 reactions
Blue	CMV LC Master	2 x 12 reactions	8 x 12 reactions
Yellow	CMV Mg-Sol [¶]	1 x 600 µl	1 x 600 µl
Red	CMV QS 1 [¶] 1 x 10 ⁴ copies/µl	1 x 200 µl	1 x 200 µl
Red	CMV QS 2 [¶] 1 x 10 ³ copies/µl	1 x 200 µl	1 x 200 µl
Red	CMV QS 3 [¶] 1 x 10 ² copies/µl	1 x 200 µl	1 x 200 µl
Red	CMV QS 4 [¶] 1 x 10 ¹ copies/µl	1 x 200 µl	1 x 200 µl
Green	CMV LC IC [¶]	1 x 1000 µl	2 x 1000 µl
White	Water (PCR grade)	1 x 1000 µl	1 x 1000 µl

[¶] QS = Quantitation Standard
IC = Internal Control
Mg-Sol = Magnesium Solution

2. Storage

The components of the *artus* CMV 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 **8.2 DNA Isolation**)
- Pipets (adjustable)
- Sterile pipet 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 for the *LightCycler 1.1/1.2/1.5* or *LightCycler 2.0* Instrument
- *LightCycler Multicolor Demo Set* (Cat. Nr. 03 624 854 001) for the installation of a *Crosstalk Color Compensation* file for the *LightCycler 2.0* Instrument
- *LightCycler* Capillaries (20 µl)
- *LightCycler* Cooling Block
- *LightCycler 1.1/1.2/1.5* (Software Version 3.5) or *LightCycler 2.0* (Software Version 4.0) Instrument
- *LightCycler* Capping Tool

4. General Precautions

The user should always pay attention to the following:

- Use sterile pipet 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

The human cytomegalovirus (CMV) is found in infected persons in blood, tissues and nearly all secretory fluids. Transmission can be oral, sexual, via blood transfusion or organ transplantation, intrauterine, or perinatal. Infection with CMV frequently leads to an asymptomatic infection followed by a lifelong persistence of the virus in the body. If symptoms occur, in teenagers or in adults, they resemble those of mononucleosis with fever, weak hepatitis, and general indisposition. Severe courses of CMV infection have been observed especially in those infected intrauterine and in immunodeficient patients.

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* CMV LC PCR Kit is a ready-to-use system for the detection of CMV DNA using polymerase chain reaction (PCR) in the *LightCycler* Instrument. The *CMV LC Master* contains reagents and enzymes for the specific amplification of a 105 bp region of the CMV genome, and for the direct detection of the specific amplicon with the *LightCycler 1.1/1.2/1.5* or *LightCycler 2.0* Instrument. In addition, the *artus* CMV LC PCR Kit contains a second heterologous amplification system to identify possible PCR inhibition.

PCR product	Selection of the fluorescence channels	
	<i>LightCycler 1.1/1.2/1.5</i> Instrument	<i>LightCycler 2.0</i> Instrument
CMV	F1	530
CMV LC IC	F3/Back-F1	705/Back 530

The amplification and detection of this *Internal Control (IC)* do not reduce the detection limit of the analytical CMV PCR (see **11.1 Analytical Sensitivity**). External positive controls (*CMV QS 1 – 4*) are supplied which allow the determination of the pathogen load. For further information, please refer to section **8.4 Quantitation**.

Attention: The temperature profile for the detection of cytomegalovirus using the *artus* CMV LC PCR Kit corresponds to the profiles of the *artus* EBV LC PCR Kit, the *artus* HSV-1/2 LC PCR Kit, and the *artus* VZV LC PCR Kit. Therefore, the PCR assays of these *artus* systems can be carried out and analyzed in one single run. Please note the recommendations on PCR analysis given in chapters **8.4 Quantitation** and **9. Data Analysis**.

8. Protocol

8.1 Pre-analytics: Specimen Collection, Storage, and Transport

Precaution: All samples have to be treated as potentially infectious material.

Attention: Current studies refer to EDTA or citrate plasma as the most suitable sample materials for CMV detection. Therefore, we recommend the use of these materials with the *artus* CMV LC PCR Kit.

The validation of the *artus* CMV LC PCR Kit has been performed using human EDTA plasma samples. Other sample materials are not validated. Please use only recommended nucleic acid isolation kits (see **8.2 DNA Isolation**) for sample preparation.

Using certain sample materials, particular instructions regarding collection, transport and storage have to be strictly observed.

8.1.1 Specimen Collection

Each blood withdrawal causes an injury of blood vessels (arteries, veins, capillaries). Only innocuous and sterile material should be used. For blood withdrawal appropriate disposables are available. For the vein puncture, too fine capillary needles should not be employed. Venous blood withdrawal should be carried out on the appropriate parts of the elbow bend, the forearm or the back of the hand. Blood has to be withdrawn with standard specimen collection tubes (red cap, Sarstedt or equivalent tube of another manufacturer). 5 – 10 ml EDTA blood should be withdrawn. Tubes should be mixed over-head directly after sample collection (8 x, do not agitate).

Attention: Samples of heparinized patients must not be used (see **8.1.4 Interfering Substances**).

8.1.2 Sample Storage

Whole blood should be separated into plasma and cellular components by centrifugation for 20 minutes at 800 – 1600 x g within six hours. The isolated plasma has to be transferred into sterile polypropylene tubes. The sensitivity of the assay can be reduced if you freeze the samples as a matter of routine or store them for a longer period of time.

8.1.3 Sample Transport

Sample material should be transported in a shatterproof transport container as a matter of principle. Thus, a potential danger of infection due to a leakage of sample can be avoided. The samples should be transported following the local and national instructions for the transport of pathogen material.*

The samples should be shipped within six hours. It is not recommended to store the samples where they have been collected. It is possible to ship the

* International Air Transport Association. Dangerous Goods Regulations, 41st Edition, 2000.704.

samples by mail, following the legal instructions for the transport of pathogen material. We recommend the sample transport with a courier. The blood samples should be shipped cooled (+2°C to +8°C) and the separated plasma deep frozen (–20°C).

8.1.4 Interfering Substances

Elevated levels of bilirubin (≤ 4.5 mg/dl), lipids (≤ 1100 mg/dl), and hemolytic samples do not influence the analytical CMV system. Heparin affects the PCR. Samples, which have been collected in tubes containing heparin as an anticoagulant, should not be used. Also, samples of heparinized patients must not be used.

8.2 DNA Isolation

The following isolation kits are recommended to isolate CMV DNA:

Sample material	Nucleic Acid Isolation Kit	Catalog Number	Manufacturer	Carrier-RNA
EDTA Plasma	QIAamp [®] DSP Virus Kit (50)	60704	QIAGEN	included
EDTA Plasma	EZ1 [®] DSP Virus Kit (48)*	62724	QIAGEN	included

*To be used in combination with the EZ1 Advanced (Cat. No. 9001411) and the EZ1 Advanced DSP Virus Card (Cat. No. 9018306) or the BioRobot[®] EZ1 DSP (Cat. No. 9001360) and the EZ1 DSP Virus Card (Cat. No. 9017707). The EZ1 DSP Virus Kit is also available as CE-IVD-marked EASYartus[®] CMV LC PCR Kits, combined with the artus CMV LC PCR Kit (Cat. Nos. EA10303 and EA10304).

- The use of **carrier RNA** is critical for the extraction efficiency and, consequently, for DNA/RNA yield. To achieve an improved stability of the carrier RNA, which is supplied with the QIAamp DSP Virus Kit and the EZ1 DSP Virus Kit, please follow the instructions for the handling and storage of the carrier-RNA in the *QIAamp DSP Virus Kit Handbook* or the *EZ1 DSP Virus Kit Handbook*.

Important: The *Internal Control* of the artus CMV LC PCR Kit can be used directly in the isolation procedure. Please make sure to include one negative plasma sample in the isolation procedure. The corresponding signal of the

Internal Control is the basis for the evaluation of the isolation (see **8.3 Internal Control**).

8.3 Internal Control

An *Internal Control (CMV 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). For this application, add the *Internal Control* to the isolation at a ratio of 0.1 µl per 1 µl elution volume. When using the QIAamp DSP Virus Kit, the DNA is eluted for example in 60 µl Buffer AVE. Hence, 6 µl of the *Internal Control* must be added. When using the EZ1 DSP Virus Kit, the *Internal Control* has to be added following the instructions in the *EZ1 DSP Virus Kit Handbook*. The quantity of *Internal Control* used depends **only** on the elution volume. The *Internal Control* and carrier RNA (see **8.2 DNA Isolation**) should be added by strictly following the instructions in the *QIAamp DSP Virus Kit Handbook* or the *EZ1 DSP Virus Kit Handbook*.

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.

To consider a purification successful, the Ct value of the *Internal Control* of a negative plasma sample which has been processed through purification has to reach the Ct value indicated in Table 1. The stated spreading is based on the variance of the instrument and the purification. A higher deviation points to a purification problem. In this case the purification has to be checked and, if necessary, validated a second time. If you have any further questions or if you encounter problems, please contact our Technical Service.

Table 1: Accepted range of Ct values of the *Internal Control* of a negative plasma sample.

Purification kit	Instrument	Fluorescence channel	Method of analysis	Ct value
QIAamp DSP Virus Kit	<i>LightCycler 1.1/1.2/1.5</i>	F3/Back-F1	<i>Second derivative maximum</i>	14 ± 3
QIAamp DSP Virus Kit	<i>LightCycler 2.0</i>	705/Back 530	<i>Auto</i>	14 ± 3
EZ1 DSP Virus Kit	<i>LightCycler 1.1/1.2/1.5</i>	F3/Back-F1	<i>Second derivative maximum</i>	15 ± 3
EZ1 DSP Virus Kit	<i>LightCycler 2.0</i>	705/Back 530	<i>Auto</i>	15 ± 3

The *Internal Control* can optionally be used **exclusively to check for possible PCR inhibition** (see Fig. 2). For this application, add 1 µl of the *Internal Control* and 2.5 µl *CMV Mg-Sol* per reaction directly to 12.5 µl *CMV LC Master*. For each PCR reaction use 15 µl of the Master Mix produced as described above* and add 10 µl of the purified sample. If you are preparing a PCR run for several samples, please increase the volume of the *CMV LC Master*, *CMV Mg-Sol* and the *Internal Control* according to the number of samples (see **8.5 Preparing the PCR**).

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

8.4 Quantitation

The enclosed *Quantitation Standards (CMV QS 1–4)* are treated as previously purified samples and the same volume is used (10 µl). To generate a standard curve on the *LightCycler* Instrument, all four *Quantitation Standards* should be used as follows:

* 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.

LightCycler 1.1/1.2/1.5 Instrument

Define the *CMV QS 1 – 4* 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).

LightCycler 2.0 Instrument

In order to define the standards, please activate the function *Analysis Type* in the menu of the window *Samples* and select *Absolute Quantification*. You can now define the *CMV QS 1 – 4* as standards and enter the corresponding concentrations for each standard (see *LightCycler Operator's Manual*, Version 4.0, Chapter 2.2 Entering Sample Information). Make sure that the function *Enable Controls* is **not** activated. Otherwise the selection of analysis options for the data analysis is restricted (see **9.2 Data Analysis of the PCR Data on the LightCycler 2.0 Instrument**).

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

Attention: In order to assure an accurate quantitation, it is highly recommended to supplement the Master Mix used for the *Quantitation Standards* with the according amount of *Internal Control*. For this purpose, add for each *Quantitation Standard (CMV QS 1 – CMV QS 4)* 1 µl of the *Internal Control* and 2.5 µl *CMV Mg-Sol* directly to 12.5 µl *CMV LC Master* (for a schematic overview see Fig. 2). This pipetting scheme is generally applicable for the *CMV Quantitation Standards* and independent from the number of *Quantitation Standards* used.

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:

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

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 1.1/1.2/1.5* or *LightCycler 2.0* 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**).

8.5 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* as well as one negative control (*Water, PCR grade*) are included per PCR run. To generate a standard curve, use all supplied *Quantitation Standards* (*CMV 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 **8.3 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	CMV LC Master	12.5 µl	150 µl
	CMV Mg-Sol	2.5 µl	30 µl
	CMV 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	10 µl	10 µl each
	Total Volume	25 µl	25 µl each

If you want to use the *Internal Control* **exclusively to check for PCR inhibition**, it must be added directly to the *CMV 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	CMV LC Master	12.5 µl	150 µl
	CMV Mg-Sol	2.5 µl	30 µl
	CMV LC IC	1 µl	12 µl
	Total Volume	16 µl*	192 µl
2. Preparation of PCR assay	Master Mix	15 µl	15 µl each
	Sample/CMV QS 1 – 4	10 µl	10 µl each
	Total Volume	25 µl	25 µl each

Pipette 15 µl of the Master Mix into the plastic reservoir of each capillary. Then add 10 µl of the eluted sample DNA. Correspondingly, 10 µl of at least one of the *Quantitation Standards (CMV QS 1 – 4)* must be used as a positive control and 10 µl of water (*Water, PCR grade*) as a negative control. Close the capillaries. In order to create the standard curve, it is highly recommended to supplement the Master Mix used for the *Quantitation Standards* with the according amount of *Internal Control* (see **8.4 Quantitation**). 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 (2000 rpm).

* 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.

Addition of the *Internal Control* to the Purification Procedure

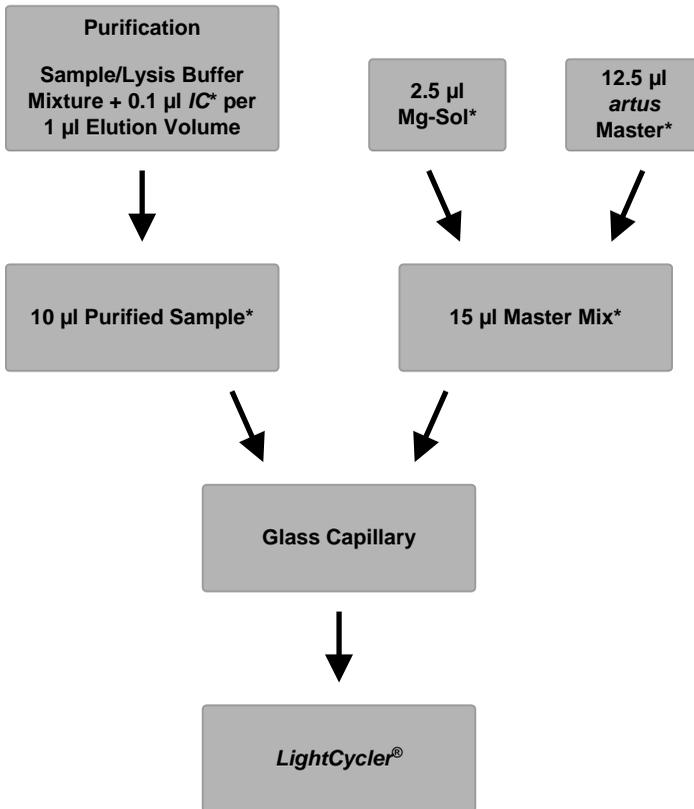


Fig. 1: Schematic workflow for the control of both the purification procedure and PCR inhibition.

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

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

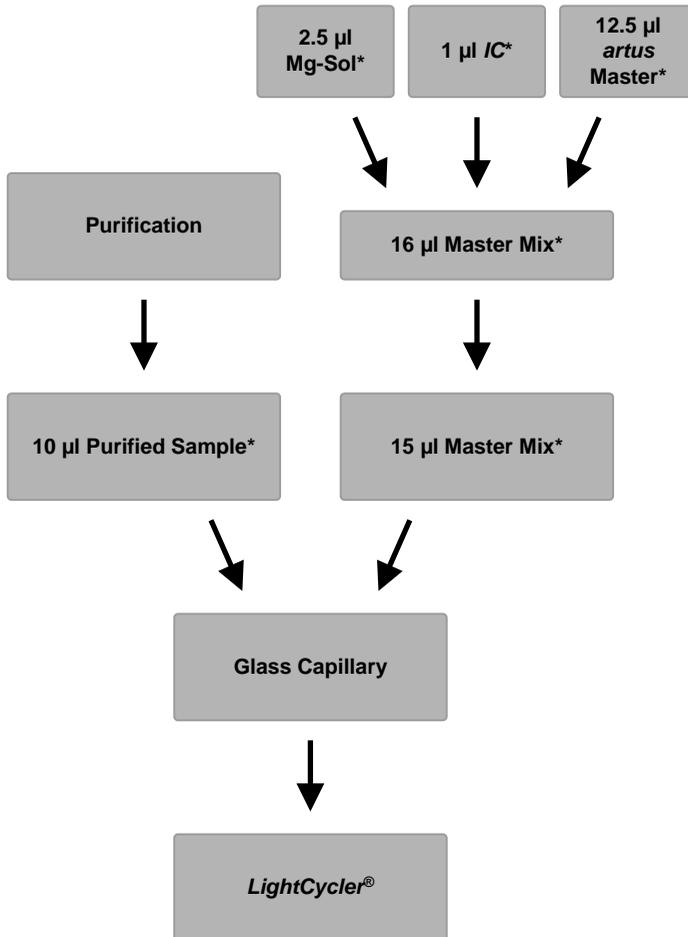


Fig. 2: Schematic workflow for the control of PCR inhibition.

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

8.6 Programming of the *LightCycler* Instruments

8.6.1 Programming the *LightCycler* 1.1/1.2/1.5 Instrument

For the detection of CMV DNA, create a temperature profile on your *LightCycler* 1.1/1.2/1.5 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 (optional) | 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* 1.1/1.2/1.5 Instrument in the *LightCycler Operator's Manual*. Step D. (Melting Curve) in the PCR program is **optional** and is only required for the differentiation of HSV1 and HSV2 when using the *artus* HSV-1/2 LC PCR Kit.

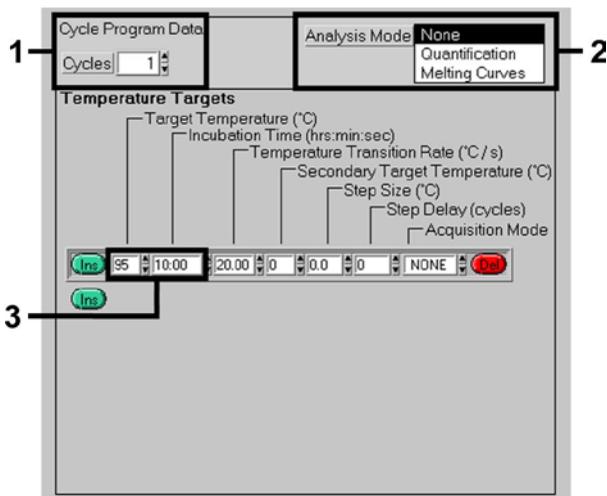


Fig. 3: Initial Activation of the Hot Start Enzyme.

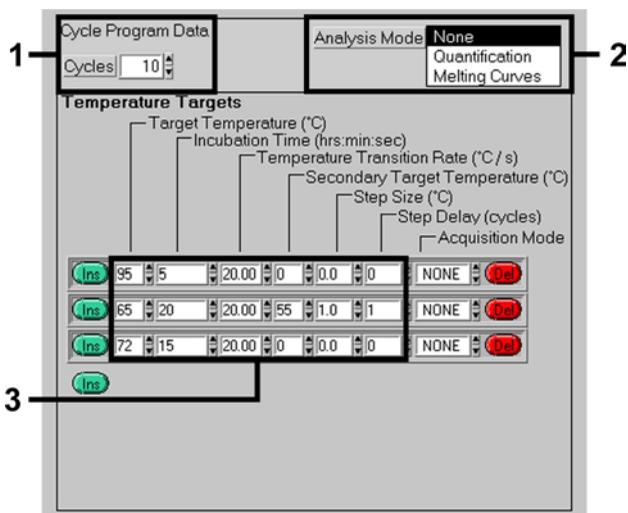


Fig. 4: Touch Down Step.

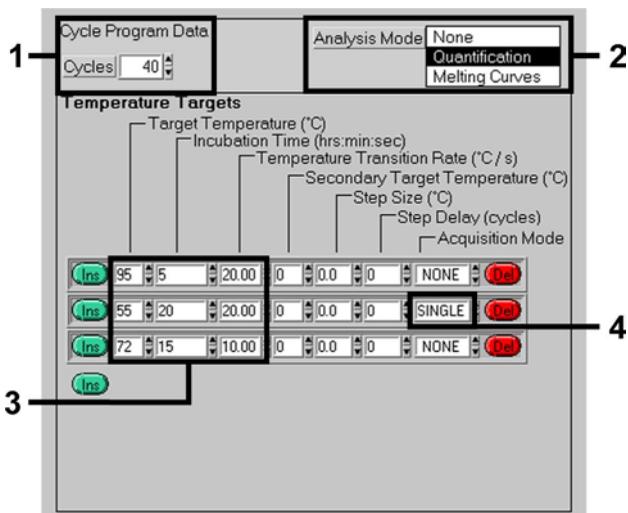


Fig. 5: Amplification of the DNA.

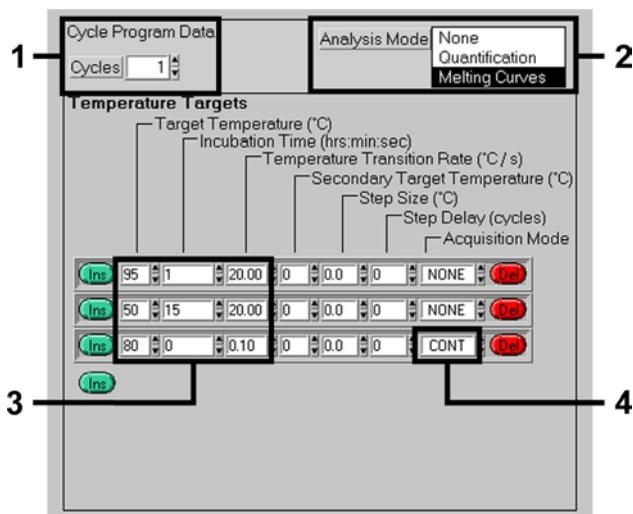


Fig. 6: Melting Curve (applicable only, if *artus* HSV-1/2 LC PCR Kit is run in parallel).

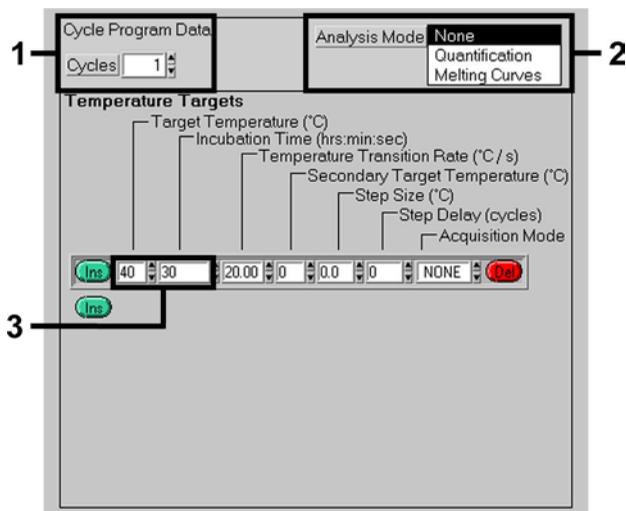


Fig. 7: Cooling.

8.6.2 Programming the *LightCycler 2.0* Instrument

To program a PCR run with *LightCycler 2.0* Instrument please activate the option *New* in the main menu and select *LightCycler Experiment*.

Subsequently, for the detection of CMV DNA, create a temperature profile on your *LightCycler 2.0* Instrument according to the following five steps (see Fig. 8 – 12).

- | | | |
|----|--|---------|
| A. | Initial Activation of the Hot Start Enzyme | Fig. 8 |
| B. | Touch Down Step | Fig. 9 |
| C. | Amplification of the DNA | Fig. 10 |
| D. | Melting Curve (optional) | Fig. 11 |
| E. | Cooling | Fig. 12 |

Step D. in the PCR program is **optional** and is only required for the differentiation of HSV1 and HSV2 when using the *artus* HSV-1/2 LC PCR Kit.

Make sure that you first enter the number of capillaries prepared for this PCR run (*Max. Seek Pos.*, see Fig. 8).

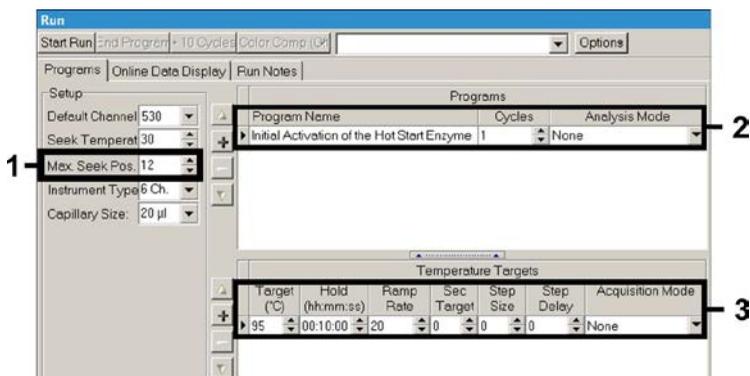


Fig. 8: Initial Activation of the Hot Start Enzyme.

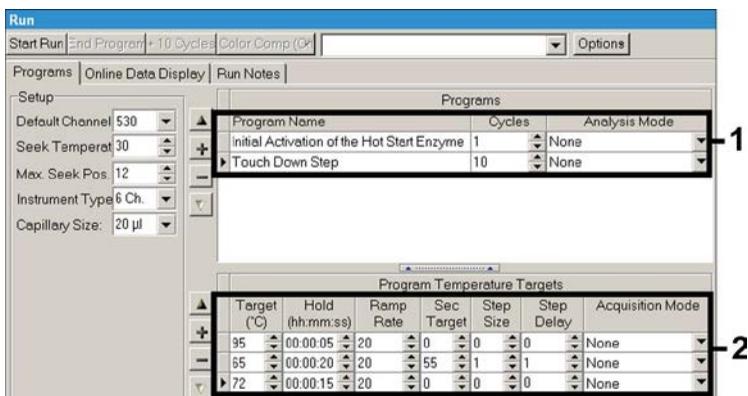


Fig. 9: Touch Down Step.

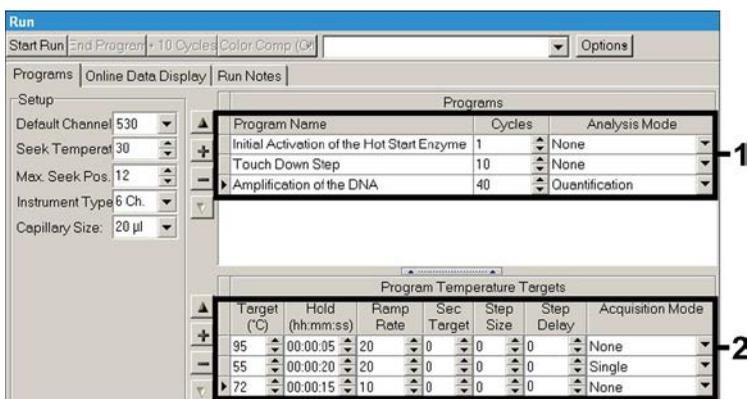


Fig. 10: Amplification of the DNA.

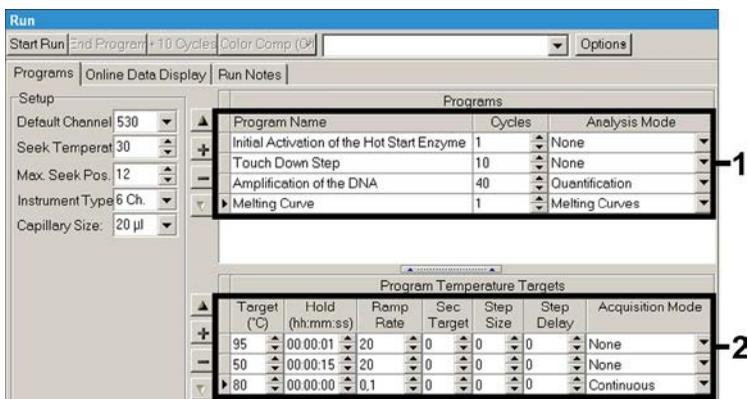


Fig. 11: Melting Curve (applicable only, if *artus* HSV-1/2 LC PCR Kit is run in parallel).

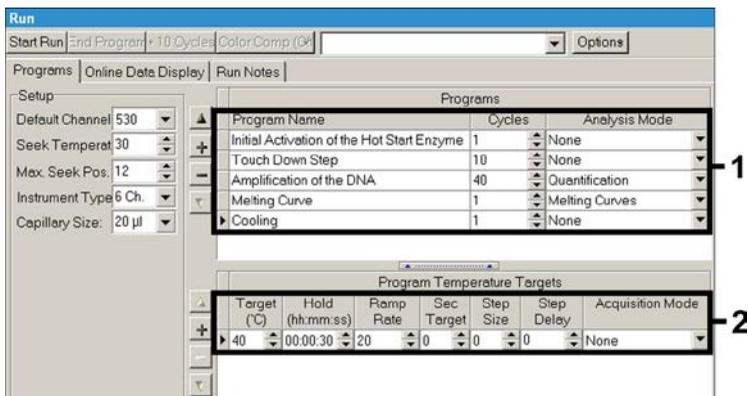


Fig. 12: Cooling.

To enter the sample specifications, please activate the button *Samples*.

- In the window *Capillary View* first enter the total number of planned PCR preparations for the PCR run (*Sample Count*).
- Then, you can assign names to the samples under *Sample Name*.
- Also select under *Selected Channels* the fluorescence channels 530 for the detection of the analytical CMV PCR and 705 for the detection of the *Internal Control* PCR.
- To define the standards and to assign the corresponding concentrations, please select the option *Absolute Quantification* under *Analysis Type* (see **8.4 Quantitation**).
- Make sure that the function *Enable Controls* is **not** activated. Otherwise the selection of analysis options for the data analysis is restricted (the mode *Fit Points* is not available, see **9.2 Data Analysis of the PCR Data on the LightCycler 2.0 Instrument**). Under *Target Name* you can assign the target sequences to be detected (CMV or *Internal Control*) in the selected fluorescence channels 530 and 705. The completion of the column *Target Name* can be facilitated with the function *Auto Copy*. To define the *Target Name* helps to get a better overview, but it is not strictly required for data analysis.
- To generate a standard curve for data analysis, the *Quantitation Standards* should be defined with their corresponding concentrations. Therefore, please select *Standard* under *Sample Type* and enter the corresponding concentration for each standard under *Concentration*.
- The programmed temperature profile can be stored on the computer's hard drive, to make use of it again for further runs. For this purpose, activate the function *Save As* under the menu *File*, upon which a new window appears. Please select under *Templates and Macros* the submenu *Run Templates* and save the data under an appropriate name.
- In order to start the PCR run, change to the field *Run* and activate the function *Start Run* (see Fig. 13). The PCR program will start after entering the location where the data should be saved.

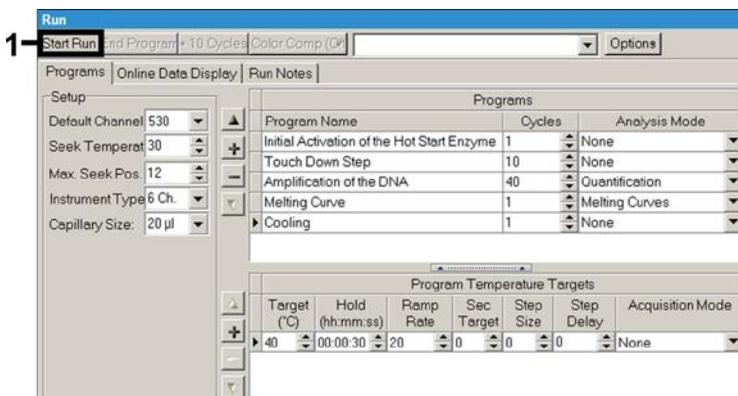


Fig. 13: Start of the PCR run.

9. Data Analysis

9.1 Data Analysis of the PCR Data on the *LightCycler* 1.1/1.2/1.5 Instrument

For the analysis of the PCR data collected with the *LightCycler* 1.1/1.2/1.5 Instrument, we recommend the use of the *LightCycler* Software Version 3.5.

In multicolor analyses, interferences occur between fluorimeter channels. The *LightCycler* 1.1/1.2/1.5 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* CMV LC PCR Kit, please select fluorescence display options F1 for the analytical CMV PCR and F3/Back-F1 for the *Internal Control* PCR, respectively. For the analysis of quantitative runs, please follow the instructions given in **8.4 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 analyze these different systems with the corresponding *Quantitation Standards* separately. Please select the carousel positions for the analysis accordingly.

The following results are possible:

1. A signal is detected in fluorimeter channel F1.

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

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

2. In fluorimeter channel 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 CMV DNA is detectable. It can be considered negative.

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

3. No signal is detected in the F1 or in 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 are given in Fig. 14 and Fig. 15.

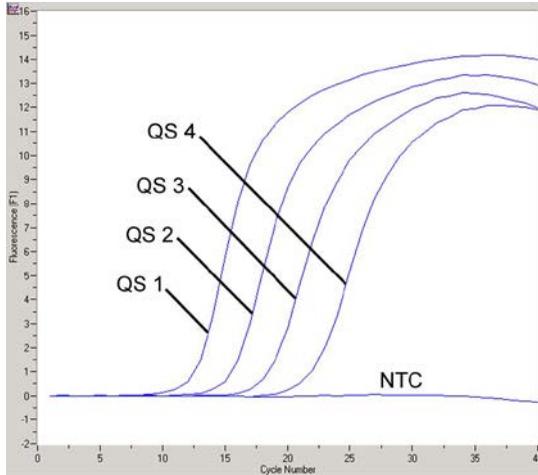


Fig. 14: Detection of the *Quantitation Standards (CMV QS 1 – 4)* in fluorimeter channel F1 of the *LightCycler 1.1/1.2/1.5* Instrument. NTC: no template control (negative control).

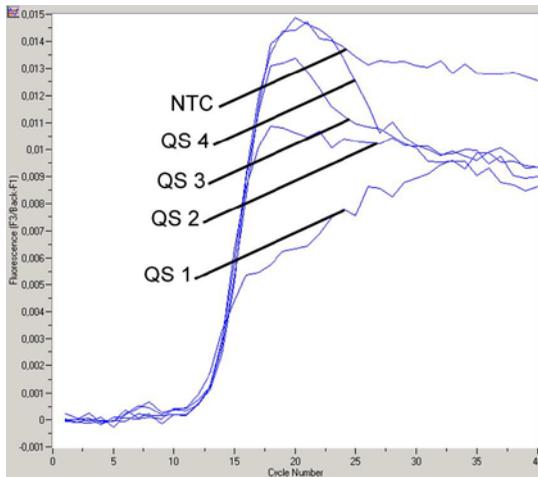


Fig. 15: Detection of the *Internal Control (IC)* in fluorimeter channel F3/Back-F1 of the *LightCycler 1.1/1.2/1.5* Instrument with simultaneous amplification of *Quantitation Standards (CMV QS 1 – 4)*. NTC: no template control (negative control).

9.2 Data Analysis of the PCR Data on the *LightCycler 2.0* Instrument

For the analysis of the PCR data collected with the *LightCycler 2.0* Instrument please use the *LightCycler* Software Version 4.0. Please consider the instructions given in the *LightCycler 2.0 Instrument Operator's Manual Version 4.0*.

For the analysis of PCR data please proceed as follows (see Fig. 16):

- Activate the function *Analysis* in the menu strip and select the option *Absolute Quantification*. As a matter of principle, all amplification data generated with the *artus* LC PCR Kit should be analyzed with this function.
- The *LightCycler* Software Version 4.0 contains a file termed *Color Compensation File*, which compensates multicolor analyses interferences between fluorescence channels. Open this file during or after the PCR run by activating the *Color Comp (On/Off)* and then the *Select Color Compensation* button (see Fig. 16). If no *Color Compensation File* is installed, generate the file according to the instructions given in the *LightCycler Operator's Manual*.
- After the *Color Compensation File* has been activated, separate signals appear in the fluorescence channels. For analysis of the PCR results gained with the *artus* CMV LC PCR Kit, please select fluorescence display options 530 for the analytical CMV PCR and 705/Back 530 for the *Internal Control* PCR, respectively.

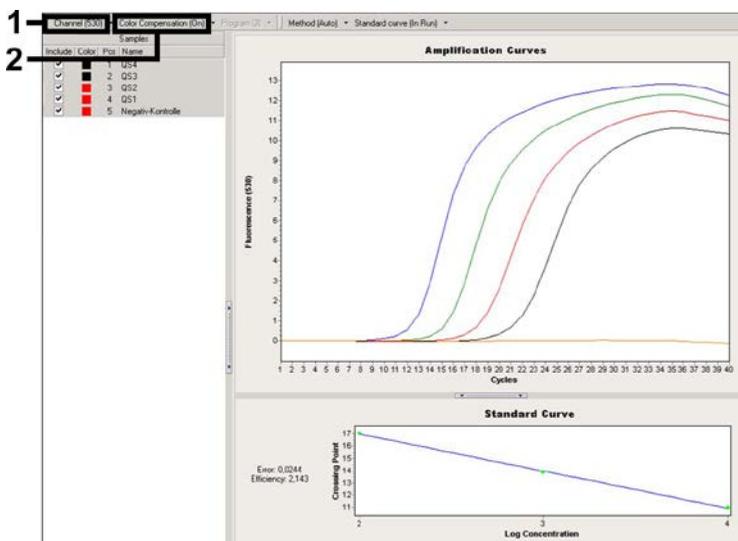


Fig. 16: Activation of the *Color Compensation File* and selection of the fluorescence channel.

For the analysis of quantitative runs, please follow the instructions given in **8.4 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.

Once the setting of analysis options is completed, the following results are possible:

1. A signal is detected in fluorescence channel 530.

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

In this case, the detection of a signal in the 705/Back 530 channel is dispensable, since high initial concentrations of CMV DNA (positive signal in the 530 channel) can lead to a reduced or absent fluorescence signal of the *Internal Control* in the 705/Back 530 channel (competition).

2. In fluorescence channel 530 no signal is detected. At the same time, a signal from the *Internal Control* appears in the 705/Back 530 channel.

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

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

3. No signal is detected in the 530 or in 705/Back 530 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 are given in Fig. 17 and Fig. 18.

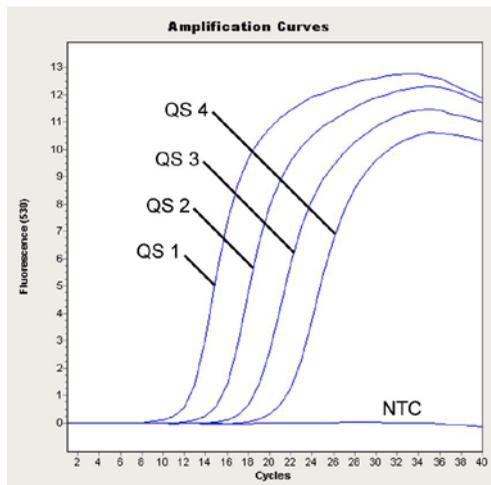


Fig. 17: Detection of the *Quantitation Standards (CMV QS 1 – 4)* in fluorescence channel 530 of the *LightCycler 2.0* Instrument. NTC: no template control (negative control).

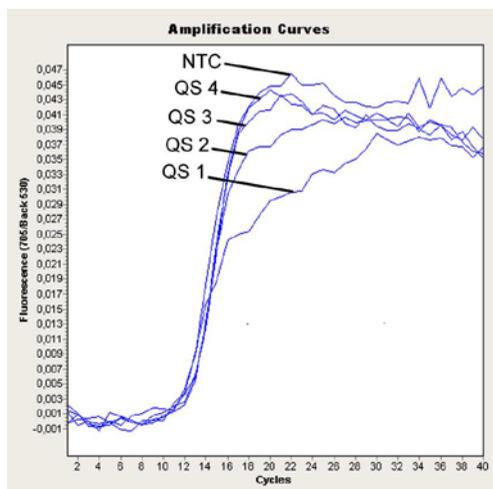


Fig. 18: Detection of the *Internal Control (IC)* in fluorescence channel 705/Back 530 of the *LightCycler 2.0* Instrument with simultaneous amplification of *Quantitation Standards (CMV QS 1 – 4)*. NTC: no template control (negative control).

10. Troubleshooting

No signal with positive controls (*CMV QS 1 – 4*) in fluorescence channel F1 or 530:

- The selected fluorescence channel for PCR data analysis does not comply with the protocol.
 - For data analysis, select the fluorescence channel F1 or 530 for the analytical *CMV PCR* and the fluorescence channel F3/Back-F1 or 705/Back 530 for the *Internal Control PCR*.
- Incorrect programming of the temperature profile of the *LightCycler 1.1/1.2/1.5* or *LightCycler 2.0* Instrument.
 - Compare the temperature profile with the protocol (see **8.6 Programming of the *LightCycler* Instrument**).
- Incorrect configuration of the PCR reaction.
 - Check your work steps by means of the pipetting scheme (see **8.5 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* CMV 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* of a negative plasma sample which has been processed through purification (deviation higher than $Ct = 14 \pm 3$ when using the QIAamp DSP Virus Kit; deviation higher than $Ct = 15 \pm 3$ when using the EZ1 DSP Virus Kit; see Table 1) and simultaneous absence of a signal in channel F1 or 530:

- 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 our recommended isolation method (see **8.2 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 **8.2 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 **8.2 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* CMV 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 fluorescence channel 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.
 - Strictly pipet the positive controls 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

The analytical detection limit as well as the analytical detection limit in consideration of the purification (sensitivity limits) were assessed for the *artus* CMV LC PCR Kit. The analytical detection limit in consideration of the purification is determined using CMV positive clinical specimens in combination with a particular extraction method. In contrast, the analytical detection limit is determined without clinical specimens and independent from the selected extraction method, using CMV DNA of known concentration.

In order to determine the **analytical sensitivity** of the *artus* CMV LC PCR Kit, a dilution series of genomic CMV DNA has been set up from 10 to nominal 0.00316 CMV copies/ μ l and analyzed on the **LightCycler 1.1/1.2/1.5 Instrument** in combination with the *artus* CMV LC PCR Kit. Testing was carried out on three different days on eight replicates. The results were

determined by a probit analysis. The analytical detection limit of the *artus* CMV LC PCR Kit in combination with the *LightCycler 1.1/1.2/1.5* Instrument is 0.49 copies/ μ l ($p = 0.05$). This means that there is a 95 % probability that 0.49 copies/ μ l will be detected.

The **analytical sensitivity in consideration of the purification (QIAamp DSP Virus Kit)** of the *artus* CMV LC PCR Kit on the *LightCycler 1.1/1.2/1.5* Instrument was determined using a dilution series from CMV virus material from 1000 to nominal 0.316 CMV copies/ml spiked in clinical plasma specimens. These were subjected to DNA extraction using the QIAamp DSP Virus Kit (extraction volume: 0.5 ml, elution volume: 60 μ l). Each of the eight dilutions was analyzed with the *artus* CMV LC PCR Kit on three different days on eight replicates. The results were determined by a probit analysis. A graphical illustration of the probit analysis is shown in Fig. 19. The analytical detection limit in consideration of the purification of the *artus* CMV LC PCR Kit in combination with the *LightCycler 1.1/1.2/1.5* Instrument is 64.9 copies/ml ($p = 0.05$). This means that there is a 95 % probability that 64.9 copies/ml will be detected.

Probit analysis: Cytomegalovirus (*LightCycler 1.1/1.2/1.5*)

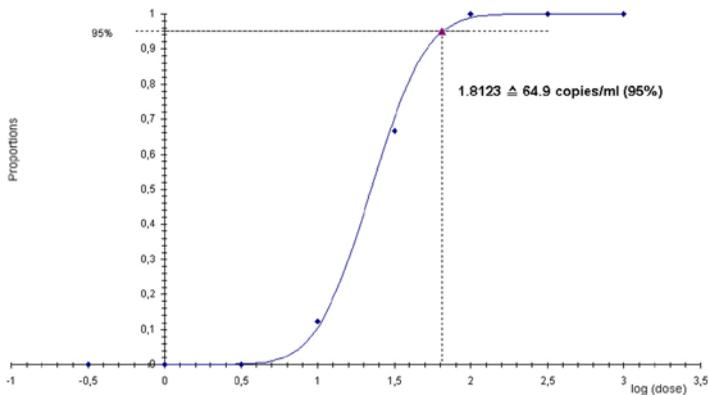


Fig. 19: Analytical sensitivity in consideration of the purification (QIAamp DSP Virus Kit) of the *artus* CMV LC PCR Kit on the *LightCycler 1.1/1.2/1.5* Instrument.

In order to determine the **analytical sensitivity** of the *artus* CMV LC PCR Kit, a dilution series of genomic CMV DNA has been set up from 10 to nominal 0.00316 CMV copies/μl and analyzed on the **LightCycler 2.0 Instrument** in combination with the *artus* CMV LC PCR Kit. Testing was carried out on three different days on eight replicates. The results were determined by a probit analysis. The analytical detection limit of the *artus* CMV LC PCR Kit in combination with the *LightCycler 2.0* Instrument is 0.65 copies/μl ($p = 0.05$). This means that there is a 95 % probability that 0.65 copies/μl will be detected.

The **analytical sensitivity in consideration of the purification (QIAamp DSP Virus Kit)** of the *artus* CMV LC PCR Kit on the **LightCycler 2.0 Instrument** was determined using a dilution series from CMV virus material from 1000 to nominal 0.316 CMV copies/ml spiked in clinical plasma specimens. These were subjected to DNA extraction using the QIAamp DSP Virus Kit (extraction volume: 0.5 ml, elution volume: 60 μl). Each of the eight dilutions was analyzed with the *artus* CMV LC PCR Kit on three different days on eight replicates. The results were determined by a probit analysis. A graphical illustration of the probit analysis is shown in Fig. 20. The analytical detection limit in consideration of the purification of the *artus* CMV LC PCR Kit in combination with the *LightCycler 2.0* Instrument is 78.9 copies/ml ($p = 0.05$). This means that there is a 95 % probability that 78.9 copies/ml will be detected.

Probit analysis: Cytomegalovirus (*LightCycler 2.0*)

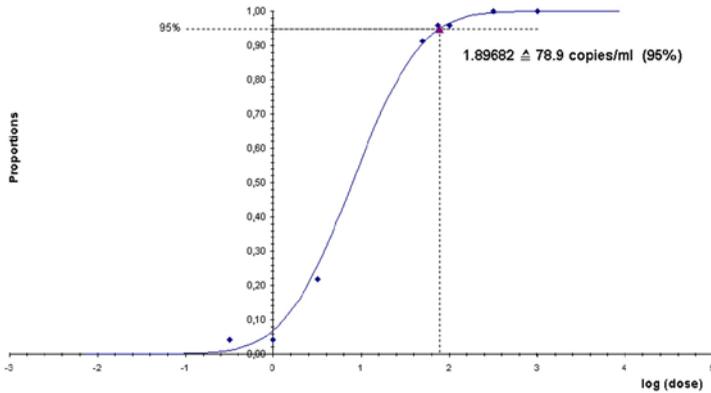


Fig. 20: Analytical sensitivity in consideration of the purification (QIAamp DSP Virus Kit) of the *artus* CMV LC PCR Kit on the *LightCycler 2.0* Instrument.

The analytical detection limit in consideration of the purification of the *artus* CMV LC PCR Kit in combination with the *LightCycler 1.1/1.2/1.5/2.0* Instrument and the EZ1 DSP Virus Kit (extraction volume: 0.4 ml, elution volume: 60 μ l) on the EZ1 Advanced or BioRobot EZ1 DSP is 67.2 copies/ml ($p = 0.05$). This means that there is a 95 % probability that 67.2 copies/ml will be detected.

11.2 Specificity

The specificity of the *artus* CMV 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 sequences published in gene banks by sequence comparison analysis. The detectability of all relevant strains has thus been ensured.

Moreover, the specificity was validated with 100 different CMV negative plasma samples. These did not generate any signals with the CMV specific primers and probes, which are included in the *CMV LC Master*.

To determine the specificity of the *artus* CMV LC PCR Kit, the control group listed in the following table (see Table 2) has been tested for cross-reactivity. None of the tested pathogens has been reactive. No cross-reactivities appeared with mixed infections.

Table 2: Testing the specificity of the kit with potentially cross-reactive pathogens.

Control group	CMV (F1)	Internal Control (F3/Back-F1)
Human herpesvirus 1 (Herpes simplex virus 1)	–	+
Human herpesvirus 2 (Herpes simplex virus 2)	–	+
Human herpesvirus 3 (Varicella-zoster virus)	–	+
Human herpesvirus 4 (Epstein-Barr virus)	–	+
Human herpesvirus 6A	–	+
Human herpesvirus 6B	–	+
Human herpesvirus 7	–	+
Human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus)	–	+
Hepatitis A virus	–	+
Hepatitis B virus	–	+
Hepatitis C virus	–	+
Human immunodeficiency virus 1	–	+
Human T cell leukemia virus 1	–	+
Human T cell leukemia virus 2	–	+
West Nile virus	–	+
Enterovirus	–	+
Parvovirus B19	–	+

11.3 Precision

The precision data of the *artus* CMV LC PCR Kit have been collected by means of the *LightCycler 1.1/1.2/1.5* Instrument and 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* CMV 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 3). In addition, precision data for quantitative results in copies/μl were determined using the corresponding Ct values (see Table 4). Based on these results, the overall statistical spread of any given sample with the mentioned concentration is 2.47% (Ct) or 14.06% (conc.), for the detection of the *Internal Control* 5.31% (Ct). These values are based on the totality of all single values of the determined variabilities.

Table 3: Precision data on basis of the Ct values.

	Standard deviation	Variance	Coefficient of variation [%]
Intra-assay variability: <i>CMV QS 4</i>	0.14	0.02	0.68
Intra-assay variability: <i>Internal Control</i>	0.04	0.00	0.32
Inter-assay variability: <i>CMV QS 4</i>	0.21	0.04	1.00
Inter-assay variability: <i>Internal Control</i>	0.14	0.02	1.18
Inter-batch variability: <i>CMV QS 4</i>	0.54	0.30	2.50
Inter-batch variability: <i>Internal Control</i>	0.72	0.52	5.73
Total variance: <i>CMV QS 4</i>	0.53	0.28	2.47
Total variance: <i>Internal Control</i>	0.64	0.41	5.31

Table 4: Precision data on basis of the quantitative results (in copies/µl).

	Standard deviation	Variance	Coefficient of variation [%]
Intra-assay variability: <i>CMV QS 4</i>	1.02	1.05	10.18
Inter-assay variability: <i>CMV QS 4</i>	1.35	1.81	13.35
Inter-batch variability: <i>CMV QS 4</i>	1.64	2.69	16.19
Total variance: <i>CMV QS 4</i>	1.42	2.02	14.06

11.4 Robustness

The verification of the robustness allows the determination of the total failure rate of the *artus* CMV LC PCR Kit. 100 CMV negative samples of plasma were spiked with CMV DNA at a final concentration of 170 copies/ml (approx. threefold concentration of the analytical sensitivity limit). After extraction using

the QIAamp DSP Virus Kit (see **8.2 DNA Isolation**) these samples were analysed with the *artus* CMV LC PCR Kit. For all CMV samples the failure rate was 0 %. In addition, the robustness of the *Internal Control* was assessed by purification and analysis of 100 CMV negative plasma samples. Thus, the robustness of the *artus* CMV LC PCR Kit is ≥ 99 %.

11.5 Reproducibility

Reproducibility data permit a regular performance assessment of the *artus* CMV LC PCR Kit as well as an efficiency comparison with other products. These data are obtained by the participation in established proficiency programs.

11.6 Diagnostic Evaluation

The *artus* CMV LC PCR Kit was evaluated in a study. Comparing the *artus* CMV LC PCR Kit to the COBAS® AMPLICOR® CMV MONITOR® Test, 177 clinical EDTA plasma specimens were analyzed retrospectively and prospectively. All specimens were pre-analyzed positive or negative using the COBAS AMPLICOR CMV MONITOR for routine diagnostics.

The specimens for testing the *artus* CMV LC PCR Kit were isolated by adding the *Internal Control* of the *artus* CMV LC PCR Kit using the QIAamp DSP Virus Kit and then analyzed by the *LightCycler Instrument*. The specimens for the COBAS AMPLICOR CMV MONITOR Test were isolated and analyzed according to the instructions of the manufacturer provided in the package insert.

All 11 samples tested positive with the COBAS AMPLICOR CMV MONITOR Test were also tested positive with the *artus* CMV LC PCR Kit. All 144 samples were tested negative with the COBAS AMPLICOR CMV MONITOR Test as well as with the *artus* CMV LC PCR Kit. 22 discordant results were obtained. The results are shown in Table 5.

Table 5: Results of the comparative validation study.

		COBAS AMPLICOR CMV MONITOR Test		
		+	-	Total
artus CMV LC PCR Kit	+	11	22	33
	-	0	144	144

If the results of the COBAS AMPLICOR CMV MONITOR Test are taken as reference, the diagnostic sensitivity of all samples of the *artus* CMV LC PCR Kit is 100%, and the diagnostic specificity is 86.7%.

Further testing of the 22 discordant samples confirmed the results of the *artus* PCR Kits. Therefore it can be assumed that the discrepancy is based on the higher sensitivity of the *artus* CMV LC PCR Kit.

12. Product Use Limitations

- 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.
- Although rare, mutations within the highly conserved regions of the viral genome covered by the kit's primers and/or probe may result in underquantitation or failure to detect the presence of the virus in these cases. Validity and performance of the assay design are revised at regular intervals.

13. Safety Information

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

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

14. Quality Control

In accordance with QIAGEN's Total Quality Management System, each lot of *artus* CMV LC PCR Kit is tested against predetermined specifications to ensure consistent product quality.

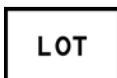
15. References

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

16. Explanation of Symbols



Use by



Batch code



Manufacturer



Catalog number



Material number



Handbook



In vitro diagnostic medical device



Components



Contains



Number



Global Trade Item Number



<N>

Contains sufficient for <N> tests



Temperature limitation



Consult instructions for use

QS

Quantitation Standard

IC

Internal Control

Mg-Sol

Magnesium Solution

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artus CMV LC PCR Kit

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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

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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

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