artus® CMV TM PCR Kit

Handbook

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

For use with the

ABI PRISM® *7000*, *7700*, and *7900HT Sequence Detection Systems*

March 2015 – Version 1

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artus CMV TM PCR Kit

For use with the *ABI PRISM 7000*, *7700*, and *7900HT Sequence Detection Systems* for the quantitative detection of CMV DNA from EDTA plasma.

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

Attention: The *artus* CMV TM PCR Kit may neither be used in combination with the *GeneAmp® 5700 SDS* nor with the 384 plate format of the *ABI PRISM 7900HT SDS*.

1. Contents

	Labelling and contents	Art. No. 4503103 24 reactions
Blue	*CMV TM Master*	2 x 12 rxns
Yellow	*CMV Mg-Sol¤*	1 x 600 µl
Red	*CMV QS 1¤* 1×10^4 *cop/µl*	1 x 200 µl
Red	*CMV QS 2¤* 1×10^3 *cop/µl*	1 x 200 µl
Red	*CMV QS 3¤* 1×10^2 *cop/µl*	1 x 200 µl
Red	*CMV QS 4¤* 1×10^1 *cop/µl*	1 x 200 µl
Green	*CMV IC¤*	1 x 1,000 µl
White	*Water* (*PCR grade*)	1 x 1,000 µl

¤ *QS   =   Quantitation Standard*
    IC   =   Internal Control
    Mg-Sol =   Magnesium Solution

2. Storage

The components of the *artus* CMV TM PCR Kit should be stored at –30°C to –15°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 **7.2 DNA Isolation**)
- Pipettes (adjustable)
- Sterile pipette tips with filters
- Vortex mixer
- Desktop centrifuge with rotor for 2 ml reaction tubes
- Centrifuge with rotor for microtitre plates (optional)
- 96-well reaction plate/reaction tubes for optical measurement with corresponding optical closing materials* (see **7.5 Preparing the PCR**)
- 96-well two-part retaining rack for use with optical reaction tubes (*96-Well Tray/Retainer Set*, Cat. No. 403 081, Applied Biosystems), see **7.5 Preparing the PCR**
- Compression pad for use with optical adhesive covers (*Optical Cover Compression Pads*, Cat. No. 4 312 639, Applied Biosystems), see **7.5 Preparing the PCR**
- Applicator for sealing of the reaction plates using optical adhesive covers (*Adhesive Seal Applicator Kit*, Cat. No. 4 333 183, Applied Biosystems)
- *ABI PRISM 7000* (Software Version 1.0.1), *7700* (Software Version 1.9.1) or *7900HT SDS* (Software Version 2.1)

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* The use of reaction tubes for optical analyses with domed caps is only permitted with the *ABI PRISM® 7700 SDS* and requires an adjustment of the exposure time (see **7.6.2 Programming the *ABI PRISM® 7700 SDS***, 7.6.2.5 Important Additional Settings).

Attention: A valid calibration of the pure dyes (*Pure Spectra Component File*) and the background (*Background Component File*) is necessary when putting the instruments into operation.

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 a cooling block.

5. Principle of Real-Time PCR

Pathogen detection by the polymerase chain reaction (PCR) is based on the amplification of specific regions of the pathogen genome. In real-time PCR the amplified product is detected via fluorescent dyes. These are usually linked to oligonucleotide probes, 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).

6. Product Description

The *artus* CMV TM PCR Kit constitutes a ready-to-use system for the detection of CMV DNA using polymerase chain reaction (PCR) in the *ABI PRISM 7000*, *7700* and *7900HT Sequence Detection System*. The *CMV TM Master* contains reagents and enzymes for the specific amplification of a 105 bp region of the CMV genome. The amplicon is detected by measuring the FAM fluorescence in the *ABI PRISM SDS*. In addition, the *artus* CMV TM PCR Kit contains a second heterologous amplification system to identify possible PCR inhibition. This is detected as an *Internal Control* (*IC*) by measuring the VIC/JOE fluorescence. The detection limit of the analytical CMV PCR is not reduced. External positive controls (*CMV QS 1 - 4*) are supplied which allow the determination of the pathogen load. For further information, please refer to section **7.4 Quantitation**.

Attention: The temperature profile for the detection of cytomegalovirus using the *artus* CMV TM PCR Kit corresponds to the profiles of the *artus* EBV TM PCR Kit and the *artus* HSV-1/2 TM 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 **7.4 Quantitation** and **8. Data Analysis**.

7. Protocol

7.1 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 TM PCR Kit.

Please use only recommended nucleic acid isolation kits (see **7.2 DNA Isolation**) for sample preparation.

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

7.1.1 Specimen Collection

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: Heparinized samples must not be used (see **7.1.4 Interfering Substances**).

7.1.2 Sample Storage

Whole blood should be separated into plasma and cellular components by centrifugation for 20 minutes at 800 - 1,600 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.

7.1.3 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 biological 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 samples by mail, following the legal instructions for the transport of biological material. The blood samples should be shipped cooled (+2°C to +8°C) and the separated plasma deep frozen (–20°C).

7.1.4 Interfering Substances

Levels of ≤ 4.5 mg/dl bilirubin and ≤ 1,100 mg/dl lipids 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.

7.2   DNA Isolation

The following isolation kit is recommended to isolate CMV DNA:

Sample Material	Nucleic Acid Isolation Kit	Catalogue Number	Manufacturer	Carrier RNA
EDTA plasma	QIAamp® DSP Virus Kit (50)	60704	QIAGEN	included

- 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, please follow the instructions for the handling and storage of the carrier-RNA ("Preparing reagents and buffers", page 16/17).
- The isolation kit includes **ethanol**-containing washing buffers. Please make sure to 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.

Important: The *Internal Control* of the *artus* CMV TM 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 **7.3 Internal Control**).

7.3   Internal Control

An *Internal Control* (*CMV 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 AE buffer. Hence, 6 µl of the *Internal Control* must be added. The quantity of *Internal Control* used depends **only** on the elution volume.

The *Internal Control* and carrier RNA (see **7.2 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.

To consider a purification successful, the Ct value of the *Internal Control* of a negative plasma sample which has been processed through purification (QIAamp DSP Virus Kit) on the *ABI PRISM 7000*, *7700* and *7900HT SDS* has to reach Ct = 27 ± 3 (*threshold ABI PRISM 7000*: 0.1, *ABI PRISM 7700* and *7900HT SDS*: 0.2). 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.

The *Internal Control* can optionally be used **exclusively to check for possible PCR inhibition** (see Fig. 2). For this application, add 2 µl of the *Internal Control* and 5 µl *CMV Mg-Sol* per reaction directly to 25 µl *CMV TM Master*. For each PCR reaction use 30 µl of the Master Mix produced as described above* and add 20 µl of the purified sample. If you are preparing a PCR run for several samples please increase the volume of the *CMV TM Master*, the *CMV Mg-Sol* and the *Internal Control* according to the number of samples (see **7.5 Preparing the PCR**).

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* 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.

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7.4 Quantitation

The enclosed *Quantitation Standards* (*CMV QS 1 - 4*) are treated as previously purified samples and the same volume is used (20 µl). To generate a standard curve on an *ABI PRISM Sequence Detection System*, all four *Quantitation Standards* should be used and defined as standards with specification of the corresponding concentrations (see **7.6 Programming the ABI PRISM SDS**). The import of standard curves from previous runs is not possible with the *ABI PRISM 7000*, *7700* and *7900HT SDS* software.

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: 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, please add for each *Quantitation Standard* (*CMV QS 1 - CMV QS 4*) 2 µl of the *Internal Control* and 5 µl *CMV Mg-Sol* directly to 25 µl *CMV TM 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:

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

Please note that as a matter of principle the <u>initial</u> 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 *ABI PRISM 7000 SDS* is provided at **www.qiagen.com/Products/ByLabFocus/MDX** (**Technical Note for quantitation on the *ABI PRISM 7000 SDS***).

7.5   Preparing the PCR

Prepare the number of required reaction tubes or a 96-well reaction plate for the scheduled reactions. Recommended materials are listed in the following table:

Article	Description	Catalogue Number	Manufacturer	Retaining Rack*	Compression Pad
96-well optical reaction plate	96-Well Optical Reaction Plate	4 306 737	Applied Biosystems	no	–
Optical adhesive covers	Optical Adhesive Covers	4 311 971	Applied Biosystems	–	yes
Optical reaction tubes	*ABI PRISM* Optical Tubes, 8 Tubes/Strip	4 316 567	Applied Biosystems	yes	–
Optical reaction tubes	MicroAmp® Optical Tubes	N8010933	Applied Biosystems	yes	–
Optical caps (flat)	*ABI PRISM* Optical Caps, 8 Caps/Strip	4 323 032	Applied Biosystems	–	no

Attention: The use of reaction tubes for optical analyses with domed caps is only permitted with the *ABI PRISM 7700 SDS* and requires an adjustment of the exposure time (see **7.6.2 Programming the *ABI PRISM 7700 SDS*, 7.6.2.5 Important Additional Settings**).

When preparing the PCR reaction, please make sure that at least one *Quantitation Standard* per PCR run as well as one negative control (*Water*,

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* If the two-part retaining rack is used, it is necessary to open the reaction tubes when inserting them into and removing them from the rack. In order to avoid contamination due to this procedure, please use the lower part of the retaining rack only.

PCR grade) are performed in parallel. To generate a standard curve, use all supplied *Quantitation Standards* (*CMV QS 1 - 4*) for each PCR run.

Attention: 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 **7.4 Quantitation**). 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 **7.3 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 TM Master*	25 µl	300 µl
	CMV Mg-Sol	5 µl	60 µl
	CMV IC	0 µl	0 µl
	Total Volume	**30 µl**	**360 µl**
2. Preparation of PCR assay	Master Mix	30 µl	30 µl each
	Sample	20 µl	20 µl each
	Total Volume	**50 µl**	**50 µl each**

If you want to use the *Internal Control* **exclusively to check for PCR inhibition**, it must be added directly to the *CMV TM 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 TM Master*	25 µl	300 µl
	CMV Mg-Sol	5 µl	60 µl
	CMV IC	2 µl	24 µl
	Total Volume	**32 µl***	**384 µl**
2. Preparation of PCR assay	Master Mix	30 µl	30 µl each
	Sample/*CMV QS 1 - 4*	20 µl	20 µl each
	Total Volume	**50 µl**	**50 µl each**

Pipette 30 µl of the Master Mix into each required reaction tube or well of the 96-well reaction plate. Subsequently add 20 µl of the eluate from the DNA isolation. Mix the solution well by repeated up and down pipetting. Close the reaction tubes with the corresponding caps or, alternatively, when using a 96-well reaction plate, with optical adhesive covers (*Optical Adhesive Covers*). Centrifuge the reaction tubes (in a storage rack for PCR tubes) or the 96-well reaction plate in a centrifuge with a microtitre plate rotor for 30 seconds at 1,780 x g (4,000 rpm) in order to collect the prepared reaction volume in the bottom of the tube or well. If such a centrifuge is not at your disposal, please make sure that both the Master Mix and the sample volume are pipetted to the bottom of the tubes or wells. Store the prepared reactions at +4°C until the *ABI PRISM SDS* Instrument is programmed (see **7.6 Programming the ABI PRISM SDS**) and subsequently transfer them into the instrument.

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* 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.

Attention:

- When using optical reaction tubes in combination with optical caps, always insert a retaining rack (*96-Well Tray/Retainer Set*) into the instrument (*ABI PRISM 7000*, *7700* and *7900HT SDS*). If the two-part retaining rack is used, it is necessary to open the reaction tubes when inserting them into and removing them from the rack. In order to avoid contamination due to this procedure, please use the <u>lower</u> part of the retaining rack only.
- Using 96-well optical reaction plates in combination with optical adhesive covers requires covering by a compression pad (*Optical Cover Compression Pads*).

Addition of the *Internal Control* to the Purification Procedure
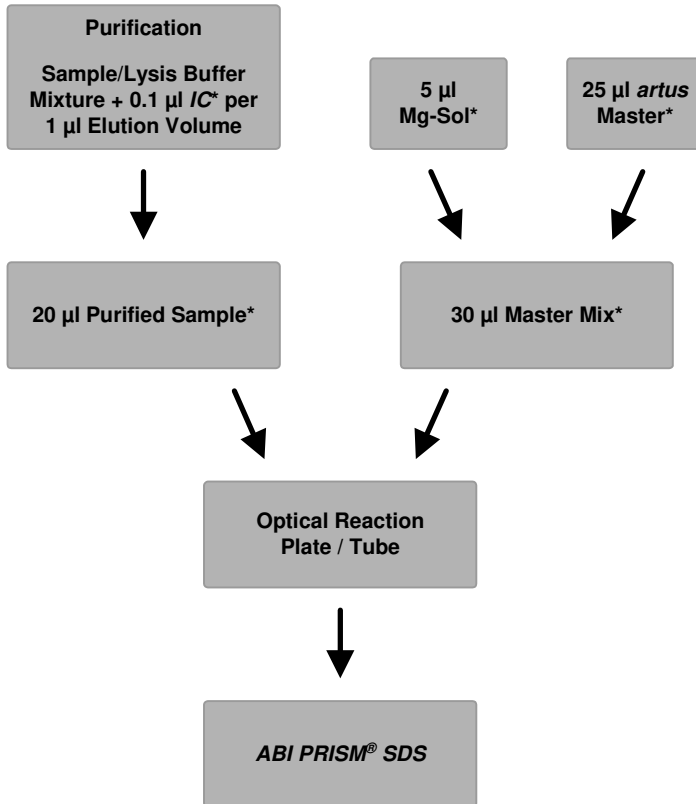
Fig. 1: Schematic workflow for the control of both the purification procedure and PCR inhibition.

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

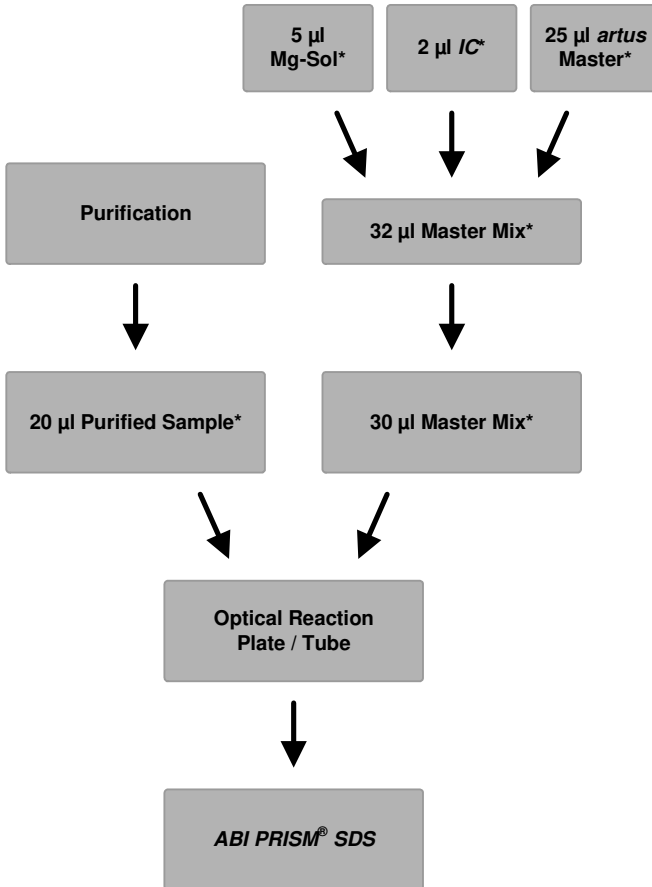**Addition of the *Internal Control* into the *artus* Master**

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

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

7.6   Programming the *ABI PRISM SDS*

The software of the *ABI PRISM 7000*, *7700* and *7900HT Sequence Detection Systems* (*SDS*) requires some additional information before starting the PCR run. The programming procedures of the instruments, however, differ considerably from each other, which is why they are treated in separate chapters as follows.

7.6.1   Programming the *ABI PRISM 7000 SDS*

For the detection of CMV DNA, create a profile on your *ABI PRISM 7000 SDS* according to the following six steps (7.6.1.1 - 7.6.1.6). All specifications refer to the *ABI PRISM 7000 SDS* software version 1.0.1. For programming details of the *ABI PRISM 7000 SDS*, please refer to the *ABI PRISM 7000 SDS User Guide*. For a better overview, the software settings are framed in bold black.

7.6.1.1   Pre-Settings for Creating a New PCR Run

Select the item *New* from the *File* menu on the *ABI PRISM 7000 SDS* and programme the following initial settings for the new document (see Fig. 3). A backup template (*SDS Template* [**.sdt*]) is available from the *Template* list or by selection using the *Browse* function (see **7.6.1.5 Saving the PCR Run**). Confirm your pre-settings (*OK*).
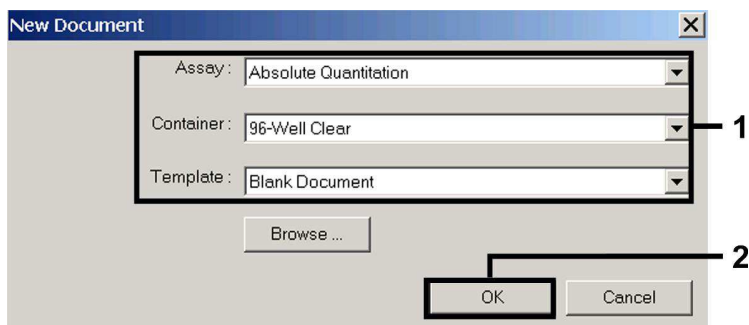

Fig. 3: Pre-settings for creating a new PCR run (*New Document*).

7.6.1.2   Creating/Selecting the Detectors

With the help of the submenu *Detector Manager* from the *Tools* menu, assign the corresponding detector dyes to the file. For the detection of CMV DNA as well as the *Internal Control* by means of the *artus* CMV TM PCR Kit, the reporters/quenchers listed in the following table are to be defined:

Detection	Reporter	Quencher
CMV DNA	FAM	none
Internal Control (*CMV IC*)	VIC	none

To create these detectors, select the option *File* (bottom left of the *Detector Manager*) and subsequently the option *New*.
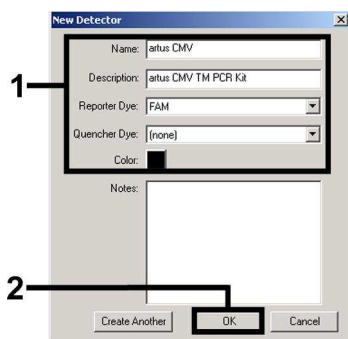
Fig. 4: Creating the CMV specific detector (*Detector Manager*).
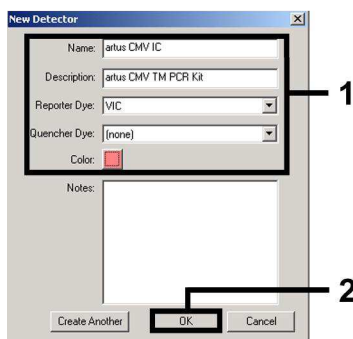
Fig. 5: Creating the *IC* specific detector (*Detector Manager*).

For the detection of CMV DNA define the reporter/quencher combination **FAM/none** in the new window. For the detection of the *Internal Control*, select the combination **VIC/none** (as shown in Fig. 4 and Fig. 5). By confirming the input data (*OK*), return to the *Detector Manager*. Mark the newly created detectors and transfer each selection to the *Well Inspector* by clicking on the option *Add to Plate Document* (see Fig. 6). Close the window (*Done*).
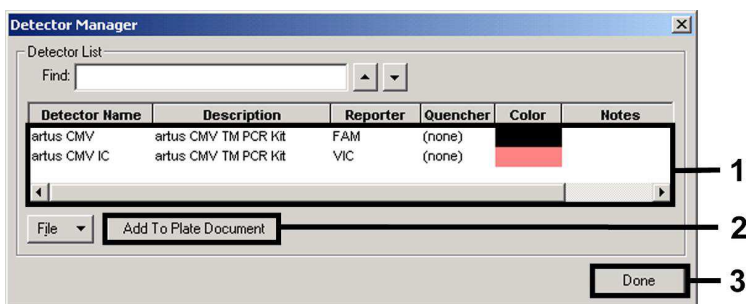
Fig. 6: Selecting the detectors (*Detector Manager*).

7.6.1.3 Assigning the Necessary Information to the Plate Positions

Open *Well Inspector* from the *View* menu to find the detectors selected under 7.6.1.2 (see Fig. 7).
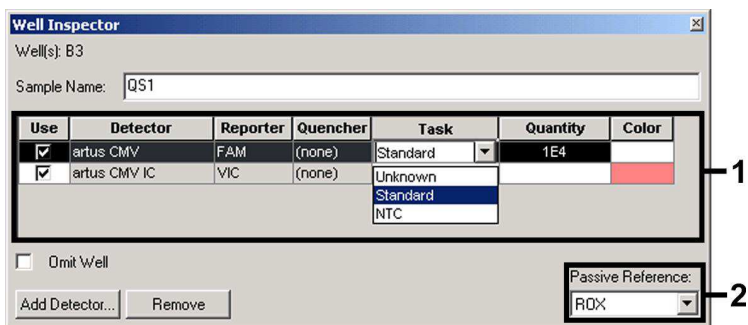

Fig. 7: Assigning the necessary information to the plate positions (*Well Inspector*).

Mark the plate positions reserved for the detection of CMV DNA. Assign the selected detectors to these positions by activating the *Use* option of both detectors, upon which a tick will appear. For the denomination of each single reaction, select the corresponding position on the plate and enter the name (*Sample Name*). Please note that preparations with an identical *Sample Name* and an identical detector assignment will be identified as replicates by the software and will be averaged with respect to the quantified pathogen load.

Then select the corresponding function (*Task*) for each sample type according to the following table:

Sample Type	Function (*Task*)	Concentration (*Quantity*)	Reporter	Quencher
Sample	Unknown	-	FAM	none
Non-template control	NTC	-	FAM	none
Standard	Standard	see **1. Contents**	FAM	none

To generate a standard curve, use all supplied *Quantitation Standards* (*CMV QS 1 - 4*) per PCR run and enter the corresponding concentrations (see **1. Contents**) for each standard (*Quantity*). Please note that for a PCR run with the *artus* CMV TM PCR Kit, **ROX** has to be set as a passive reference (*Passive Reference*). The equal distribution of the ROX dye in all PCR preparations of a lot by mixing of the *CMV TM Master* guarantees the recognition and calculation of tube-to-tube variations (fluorescence differences between several PCR preparations) by means of the *Sequence Detection Software* (normalization).

7.6.1.4   Creating the Temperature Profile

To create a temperature profile, switch from the *Setup* level to the *Instrument* level in the software. Enter the temperature profile specific for the detection of CMV DNA according to Fig. 8. In order to remove the 50°C step stored in the pre-settings, mark it with the left mouse button simultaneously holding the shift key, and subsequently delete it by using the backspace key. Make sure that the reaction volume is set to 50 µl. The option *9600 Emulation* should be activated, the pre-settings of the *Auto Increment* should remain unchanged (*Auto Increment*: 0.0°C, 0.0 Seconds).
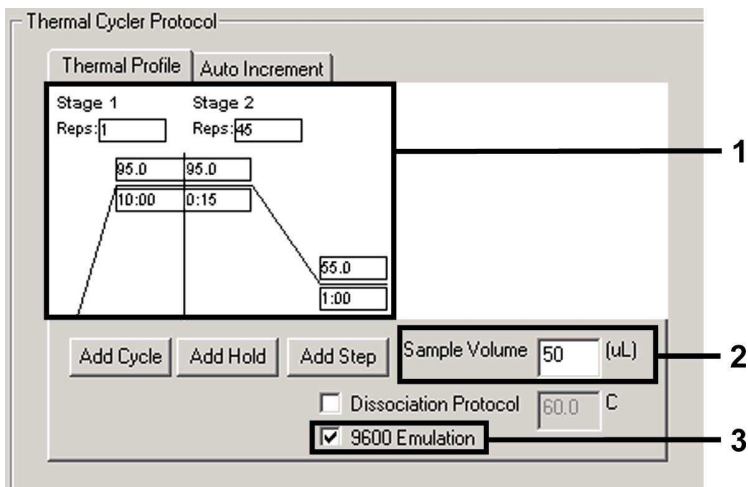
Fig. 8: Creating the temperature profile.

7.6.1.5 Saving the PCR Run

Save the settings (*Setup*) as a template in order to make use of them again later in a modified or unchanged form. By saving the settings as a *SDS Template* (**.sdt*) in the *Template Directory* (*Local Disk* [*C:*]*\Program Files\ABI PRISM 7000\Templates*, created by Applied Biosystems), this file may be selected directly in the *New Document* window from the *Template* drop-down list. Copies stored in other folders have to be opened via *Browse*. Before starting the PCR run, save it again as a *SDS Document* (**.sds*), in order to guarantee the saving of the data that will be collected during the course of the PCR.

7.6.1.6 Starting the PCR Run

Start the PCR run by selecting the option *Start* from the menu item *Instrument* or the field *Start* on the *Instrument* level.

7.6.2   Programming the *ABI PRISM 7700 SDS*

For the detection of CMV DNA, create a profile on your *ABI PRISM 7700 SDS* according to the following seven steps (7.6.2.1 - 7.6.2.7). All specifications refer to the *ABI PRISM 7700 SDS* software version 1.9.1. For programming details of the *ABI PRISM 7700 SDS*, please refer to the *ABI PRISM 7700 SDS User`s Manual*. For a better overview, the software settings are framed in bold black.

7.6.2.1   Pre-settings for Creating a New PCR Run

Select the item *New Plate* from the *File* menu on the *ABI PRISM 7700 SDS* and programme the following initial settings for the new document (see Fig. 9). Confirm the pre-settings (*OK*).
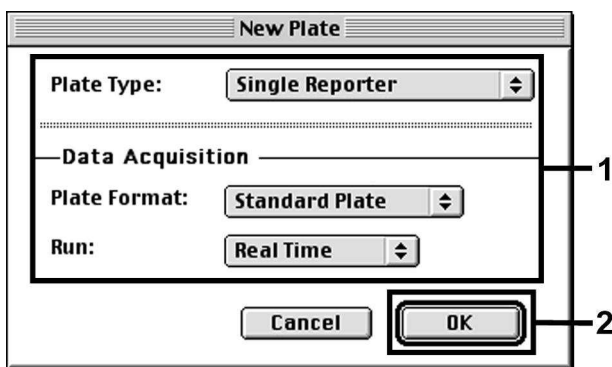

Fig. 9: Pre-settings for creating a new PCR run (*New Plate*).

7.6.2.2   Selecting the Fluorescence Dyes and Assigning the Sample Type

With the help of the *Sample Type Setup* (*Setup* level: *Sample Type/Sample Type Setup*) assign the corresponding detector dyes and the corresponding sample type to the file. For the detection of CMV DNA as well as the *Internal Control* by means of the *artus* CMV TM PCR Kit, the reporters/quenchers listed in the following table are to be defined:

Detection	Reporter	Quencher
CMV DNA	FAM	none
Internal Control (*CMV IC*)	JOE	none

For the analysis of CMV DNA by means of the *artus* CMV TM PCR Kit, select the reporter dye **FAM** as given in the table. This applies to standards (STND) and samples (UNKN) as well as to non-template controls (UNKN). For the analysis of the *Internal Control* (IPC+), define **JOE** as reporter. As quencher set **none**. The assignment of the dyes and sample types in the window *Sample Type Setup* is shown in Fig. 10.
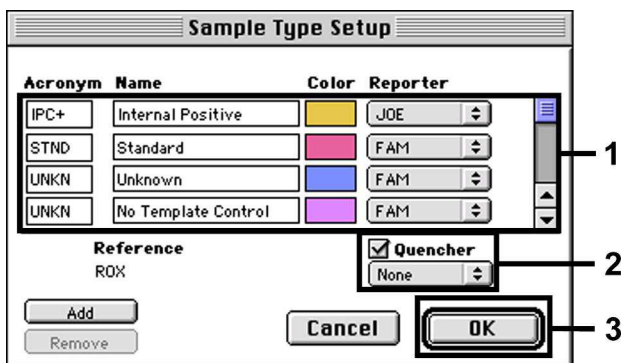
Fig. 10: Selecting the fluorescence dyes and assigning the sample type (*Sample Type Setup*).

Please assign the sample type to a corresponding function (*Acronym*) according to the following table:

Sample Type	Function (*Acronym*)	Concentration (*Quantity*)	Reporter	Quencher
Sample	UNKN	–	FAM	none
Non-template control	UNKN	–	FAM	none
Standard	STND	see **1. Contents**	FAM	none

7.6.2.3 Assigning the Necessary Information to the Plate Positions

For the assignment of the detectors and sample types to the individual plate positions, select the corresponding fields. Then open the dialogue window *Dye Layer* on the *Setup* level and assign the corresponding reporter. Upon activation of the pop-up menu *Sample Type*, you will find the sample types assigned to the reporter in the *Sample Type Setup* in the appearing list (see Fig. 11). Select the adequate sample type (see Table under 7.6.2.2) and assign the remaining plate positions by means of the *Dye Layers* and the *Sample Type* menu. A name may be assigned to each sample in the field *Sample Name*. Fields defined as *Replicates* (entering of the name of the reference sample into the *Replicate* column) are averaged by the software with respect to the quantified pathogen load and the standard deviation will be calculated.
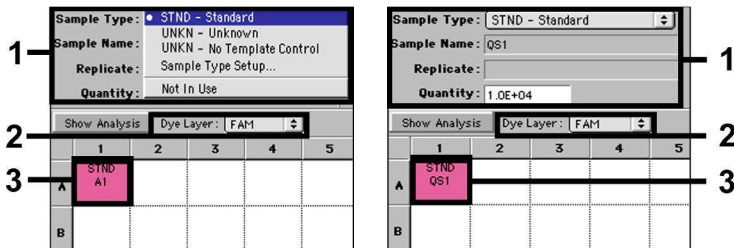

Fig. 11/12: Assigning the necessary information to the plate positions.

To generate a standard curve, use all supplied *Quantitation Standards* (*CMV QS 1 - 4*) per PCR run and enter the corresponding concentrations (see **1. Contents**) for each standard into the field *Quantity* (see Fig. 12). This is only possible, however, if the positions reserved for standards were defined as such before by means of the *Sample Type* menu.

7.6.2.4 Creating the Temperature Profile

To create a temperature profile, switch to the *Thermal Cycler Conditions* menu on the *Setup* level. Enter the temperature profile specific for the detection of CMV DNA according to Fig. 13. Make sure that the reaction volume is set to

50 µl. The pre-settings of the *Ramp* times and of the *Auto Increment* remain unchanged (*Ramp Time*: 0:00, *Auto Increment*: 0.0°C, 0.0 Seconds).
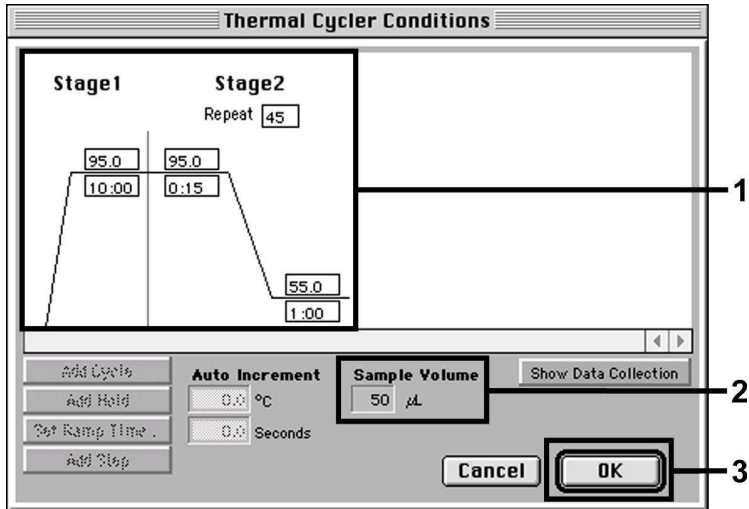

Fig. 13: Creating the temperature profile.

In addition, the *Thermal Cycler Conditions* menu contains the option *Show Data Collection*. By selecting this option the window depicted in Fig. 14 is opened. Each ramp and each plateau temperature shows a *Data Collection Icon*, which illustrates the data being collected at this stage of the run. Remove all symbols except the one for the *Annealing* step (*Stage2/*Step2) in order to exclude unnecessary fluorescence measurements. The total run time and the data quantity will thus be kept at a minimum.
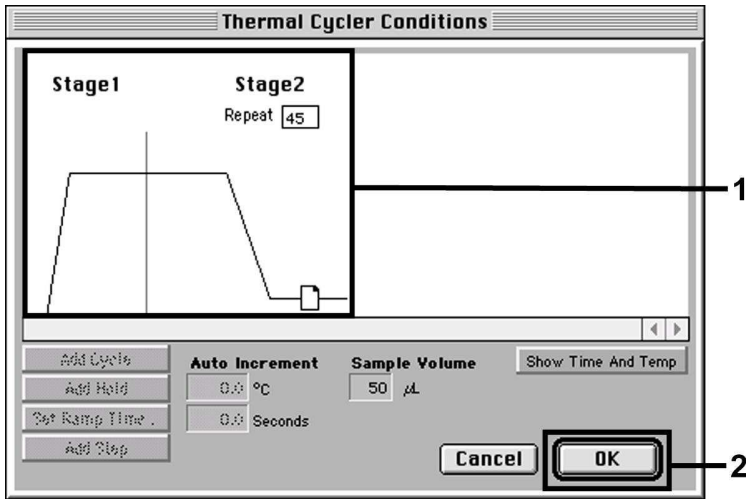
Fig. 14: Data collection.

7.6.2.5 Important Additional Settings

For the setting of the exposure time (excitation of the fluorescence dyes) as well as for the selection of the *Pure Spectra/Background* files, switch from the *Setup* level to the *Analysis* level. Select the activated sub-item *Advanced Options* to be found in the *Instrument* menu under *Diagnostics*. Adjust the settings according to Fig. 15. By inactivating the optional function *Spectra Components* (*Analysis*) the actual calibration files stored in the file *Spectra Components* at the moment of data generation are automatically used when re-evaluating already analysed runs. For an analysis of previous runs by using newly entered *Spectra Components*, activate these two fields. Please note that for a PCR run with the *artus* CMV TM PCR Kit, **ROX** has to be set as a passive reference (*Reference*). The equal distribution of the ROX dye in all PCR preparations of a lot by mixing of the *CMV TM Master* guarantees the recognition and calculation of tube-to-tube variations (fluorescence differences between several PCR preparations) by means of the *Sequence Detection Software* (normalization).

Attention: When using 96-well reaction plates for optical measurements in combination with optical adhesive covers or optical reaction tubes with flat caps, the exposure time is ten milliseconds. If you use **optical reaction tubes with domed caps**, you have to adjust this exposure time to **25 milliseconds**.
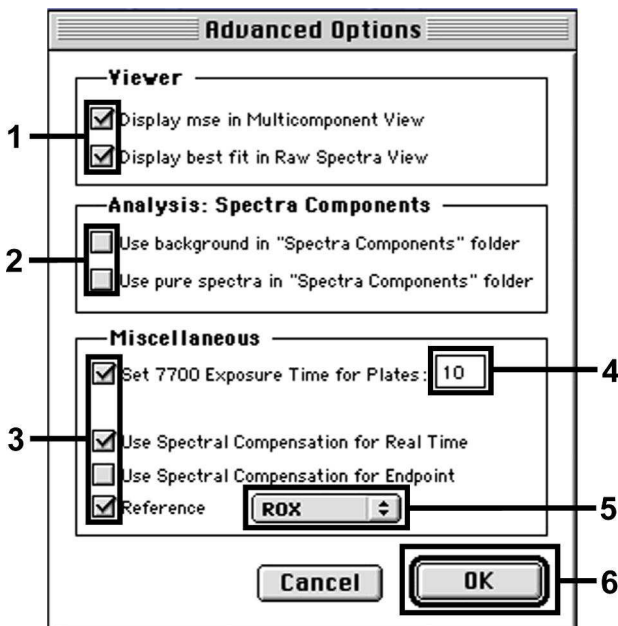

Fig. 15: Important additional settings (*Advanced Options*).

7.6.2.6 Saving the PCR Run

Save the settings (*Setup*) as a template in order to make use of them again later in a modified or unchanged form. For this purpose store the file in the *Stationary File Format*. Before starting a newly programmed PCR run, please save it again in the *Normal File Format* in order to guarantee the saving of data that will be collected during the course of the PCR.

7.6.2.7 Starting the PCR Run

Start the PCR run by selecting the option *Run* from the menu item *Instrument* or the field *Run* on the *Analysis* level.

7.6.3 Programming the *ABI PRISM 7900HT SDS*

For the detection of CMV DNA, create a profile on your *ABI PRISM 7900HT SDS* according to the following six steps (7.6.3.1 - 7.6.3.6). All specifications refer to the *ABI PRISM 7900HT SDS* software version 2.1. For programming details of the *ABI PRISM 7900HT SDS*, please refer to the *ABI PRISM 7900HT SDS User Guide*. For a better overview, the software settings are framed in bold black.

7.6.3.1 Pre-Settings for Creating a New PCR Run

Select the item *New* from the *File* menu on the *ABI PRISM 7900HT SDS* and programme the following initial settings for the new document (see Fig. 16). A backup template (*ABI PRISM SDS Template Document* [*.sdt]) is available from the *Template* list or by selection using the *Browse* function (see **7.6.3.5 Saving the PCR Run**). Confirm the pre-settings (*OK*).

Attention: The *artus* CMV TM PCR Kit may not be used in combination with the 384 plate format of the *ABI PRISM 7900HT SDS*.
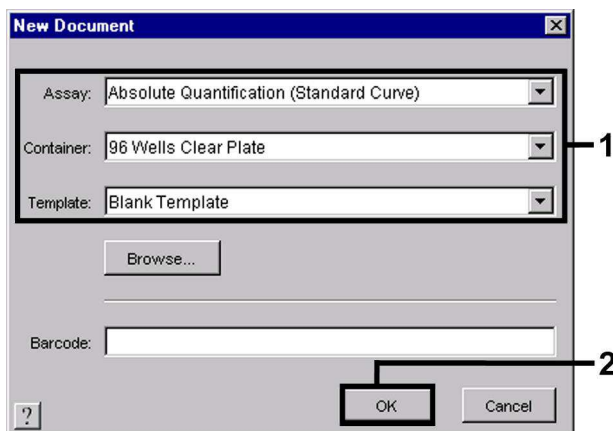

Fig. 16: Pre-settings for creating a new PCR run (*New Document*).

7.6.3.2   Creating/Selecting the Detectors

With the help of the submenu *Detector Manager* from the *Tools* menu (alternatively: *Setup* level/*Add Detector* select function), assign the corresponding detector dyes to the file. For the detection of CMV DNA as well as the *Internal Control* by means of the *artus* CMV TM PCR Kit, the reporters/quenchers listed in the following table are to be defined:

Detection	Reporter	Quencher
CMV DNA	FAM	Non Fluorescent
Internal Control (*CMV IC*)	VIC	Non Fluorescent

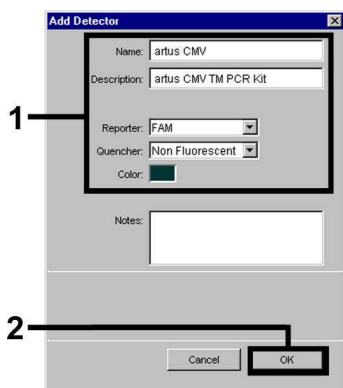To create these detectors, select the option *New* (bottom left of the *Detector Manager*).

Fig. 17: Creating   the   CMV specific detector (*Detector Manager*).
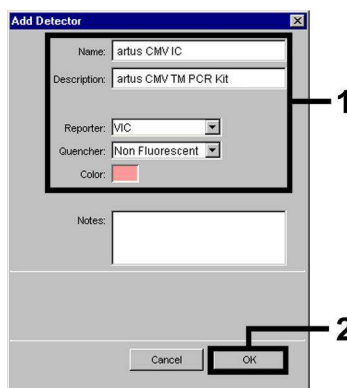
Fig. 18: Creating the *IC* specific detector (*Detector Manager*).

For the detection of CMV DNA define the reporter/quencher combination **FAM/Non Fluorescent** in the new window. For the detection of the *Internal Control*, select the combination **VIC/Non Fluorescent** (as shown in Fig. 17 and Fig. 18). By confirming the input data (*OK*), return to the *Detector Manager*. Mark the newly created detectors and transfer each selection to the *Setup* level by clicking on the option *Copy to Plate Document* (see Fig. 19). Close the window (*Done*).
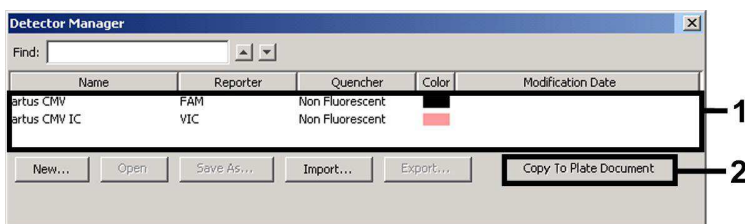
Fig. 19: Selecting the detectors (*Detector Manager*).

7.6.3.3 Assigning the Necessary Information to the Plate Positions

After having closed the *Detector Manager* (*Done*) the detectors selected under 7.6.3.2 are found on the *Setup* level listed in a table (see Fig. 20).
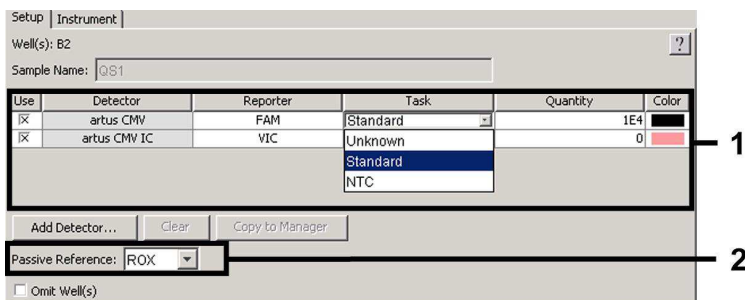

Fig. 20: Assigning the necessary information to the plate positions.

Mark the plate positions reserved for the detection of CMV DNA. Assign the selected detectors to these positions by activating the *Use* option of both detectors, upon which a cross will appear. For the denomination of each single reaction, select the corresponding position on the plate and enter the name (*Sample Name*). Please note that preparations with an identical *Sample Name* and an identical detector assignment will be identified as replicates by the software and will be averaged with respect to the quantified pathogen load. Then select the corresponding function (*Task*) for each sample type according to the following table:

Sample Type	Function (*Task*)	Concentration (*Quantity*)	Reporter	Quencher
Sample	Unknown	-	FAM	Non Fluorescent
Non-template control	NTC	-	FAM	Non Fluorescent
Standard	Standard	see **1. Contents**	FAM	Non Fluorescent

To generate a standard curve, use all supplied *Quantitation Standards* (*CMV QS 1 - 4*) per PCR run and enter the corresponding concentrations (see **1. Contents**) for each standard (*Quantity*). Please note that for a PCR run with the *artus* CMV TM PCR Kit, **ROX** has to be set as a passive reference (*Passive Reference*). The equal distribution of the ROX dye in all PCR preparations of a lot by mixing of the *CMV TM Master* guarantees the recognition and calculation of tube-to-tube variations (fluorescence differences between several PCR preparations) by means of the *Sequence Detection Software* (normalization).

7.6.3.4   Creating the Temperature Profile

To create a temperature profile, switch from the *Setup* level to the *Instrument* level in the software. Enter the valid temperature profile for the detection of CMV DNA according to Fig. 21. Make sure that the reaction volume is set to 50 µl. The option *9600 Emulation* should be activated, the pre-settings of the *Ramp* time and the *Auto Increment* should remain unchanged (*Ramp Time*: 0:00, *Auto Increment*: 0.0°C, 0.0 Seconds).
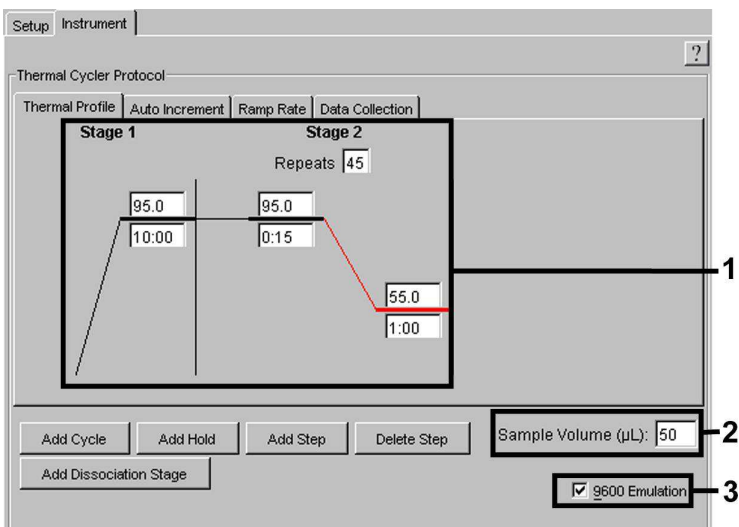
Fig. 21: Creating the temperature profile.

In addition, the *Instrument* level contains the option *Data Collection*. By selecting this option the window depicted in Fig. 22 is opened. Each ramp and each plateau temperature shows a *Data Collection Icon*, which illustrates the data being collected at this stage of the run. Remove all symbols except the one for the *Annealing* step (*Stage2/*Step2) by clicking on them in order to exclude unnecessary fluorescence measurements. The total run time and the data quantity will thus be kept at a minimum.
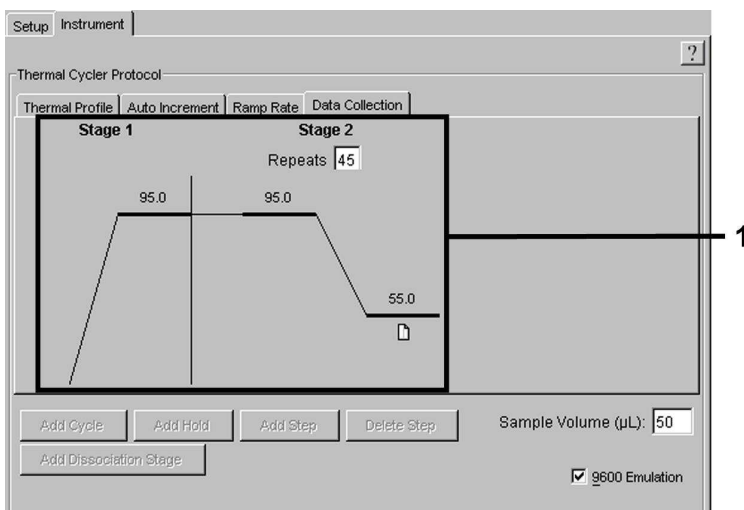
Fig. 22: Data collection.

7.6.3.5   Saving the PCR Run

Save the settings (*Setup*) as a template in order to make use of them again later in a modified or unchanged form. By saving the settings as an *ABI PRISM SDS Template Document* (**.sdt*) in the *Template Directory* ([*D:*]*Program Files\Applied Biosystems\SDS 2.1\Templates*, created by Applied Biosystems), this file may be selected directly in the *New Document* window from the *Template* list. Copies stored in other folders have to be opened via *Browse*. Before starting the PCR run, save it again as an *ABI PRISM SDS Document* (**.sds*) in order to guarantee the saving of the data that will be collected during the course of the PCR.

7.6.3.6   Starting the PCR Run

Start the PCR run by selecting the option *Start* from the menu item *Instrument*.

8. Data Analysis

A valid calibration of the dyes (**Pure Spectra Component File**) and the background (**Background Component File**) is necessary when putting the instruments into operation. These calibration files are required for the exact calculation of the results as follows:

All interfering signals generated by the instruments which influence the measurement are eliminated by the *Sequence Detection Software* of the *ABI PRISM Sequence Detection Systems* by means of the *Background Component File*.

Furthermore, interferences appear during multicolour analyses between the emission spectra of the single fluorescence dyes. The software of the *ABI PRISM SDS* compensates these interferences by calculations using the spectrum data of the individual dyes stored in the *Pure Spectra Component File*. The software uses the same file for the assignment of the fluorescence data comprising the entire measurable spectrum collected in the course of the PCR to the programmed detectors. The fluorescence data of the individual dyes are subsequently divided by the value obtained for the signal of the passive reference (ROX) in order to account for tube-to-tube variations (fluorescence differences between several PCR preparations). The thus normalized signals may then be evaluated by means of the *Amplification Plot*.

The calibration files used for the evaluation of a PCR run are automatically stored with the saving of the run. If no **calibration files** are installed, please create these files according to the instructions given in the *ABI PRISM SDS User Guide/Manual*.

If more than one *artus* TM PCR system is integrated in your PCR run (**note temperature profile**), these assays should be analysed separately. Samples with an identical *Sample Name* and an identical detector assignment will automatically be identified as replicates by the *ABI PRISM 7000* and *7900HT SDS Software* and will be averaged with respect to the quantified pathogen load.

For the analysis of quantitative runs, please follow the instructions given in **7.4 Quantitation** and in the **Technical Note for quantitation on the ABI PRISM 7000 SDS** 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. Please select the sample positions for the analysis accordingly.

The following results are possible:

1. A FAM fluorescence signal is detected.

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

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

2. No FAM fluorescence signal is detected. At the same time, a VIC/JOE fluorescence signal from the *Internal Control* appears.

   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. Neither a FAM fluorescence signal nor a VIC/JOE fluorescence signal is detected.

   No result can be concluded.

   Information regarding error sources and their solution can be found in **9. Troubleshooting**.

Examples of positive and negative PCR reactions are given in Fig. 23 / 24 (**ABI PRISM 7000 SDS**), 25 / 26 (**ABI PRISM 7700 SDS**) and 27 / 28 (**ABI PRISM 7900HT SDS**).
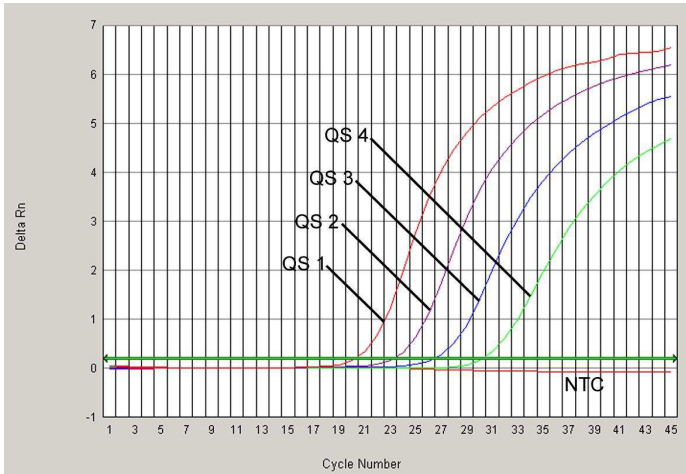
Fig. 23: Detection of the *Quantitation Standards* (*CMV QS 1 - 4*) by measurement of the FAM fluorescence signal (**ABI PRISM 7000 SDS**). NTC: non-template control (negative control).
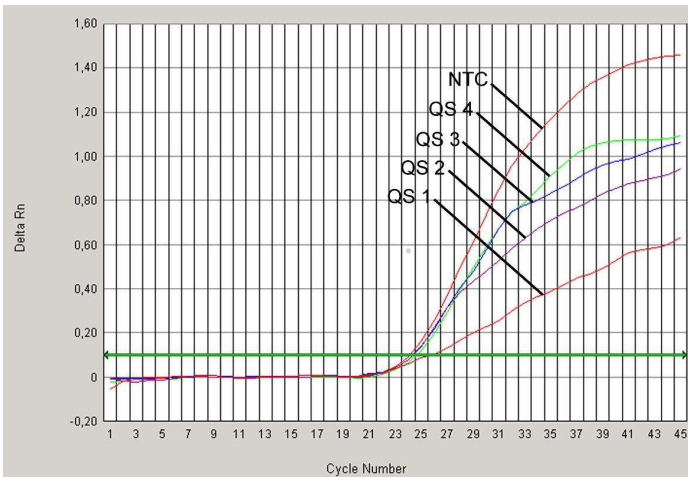

Fig. 24: Detection of the *Internal Control* (*IC*) by measurement of the VIC fluorescence signal (**ABI PRISM 7000 SDS**) with simultaneous amplification of the *Quantitation Standards* (*CMV QS 1 - 4*). NTC: non-template control (negative control).
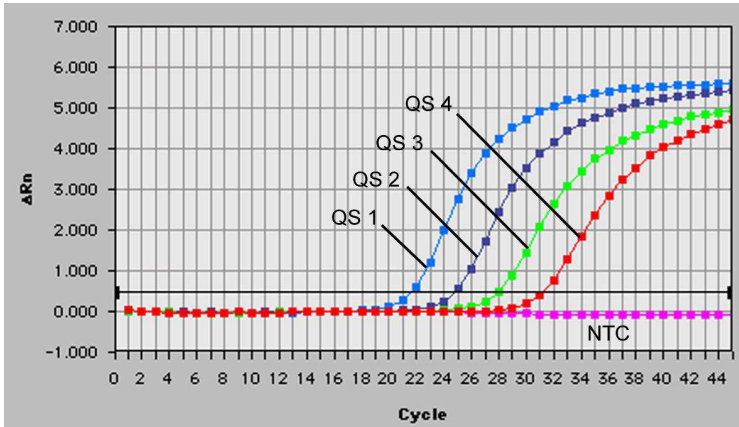
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Fig. 25: Detection of the *Quantitation Standards* (*CMV QS 1 - 4*) by measurement of the FAM fluorescence signal (**ABI PRISM 7700 SDS**). NTC: non-template control (negative control).
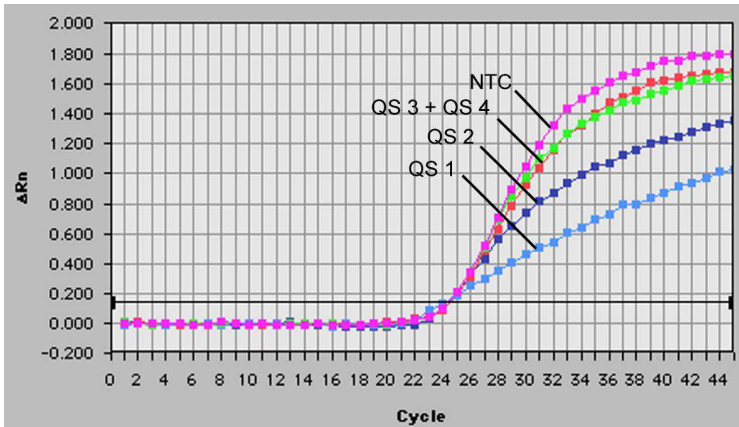

Fig. 26: Detection of the *Internal Control* (*IC*) by measurement of the JOE fluorescence signal (**ABI PRISM 7700 SDS**) with simultaneous amplification of the *Quantitation Standards* (*CMV QS 1 - 4*). NTC: non-template control (negative control).
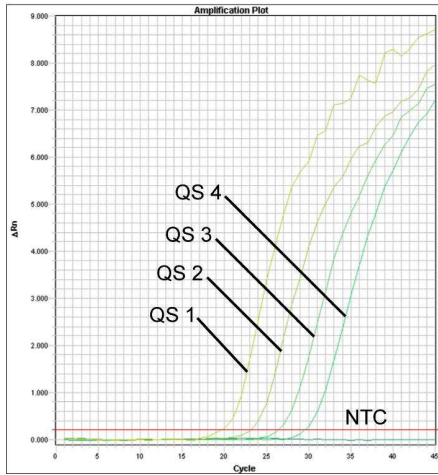
Fig. 27: Detection of the *Quantitation Standards* (*CMV QS 1 - 4*) by measurement of the FAM fluorescence signal (**ABI PRISM 7900HT SDS**). NTC: non-template control (negative control).
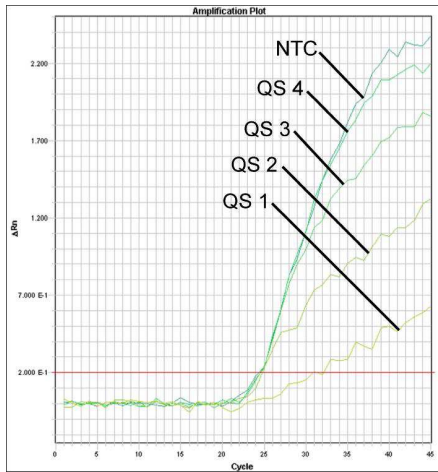

Fig. 28: Detection of the *Internal Control* (*IC*) by measurement of the VIC fluorescence signal (**ABI PRISM 7900HT SDS**) with simultaneous amplification of the *Quantitation Standards* (*CMV QS 1 - 4*). NTC: non-template control (negative control).

9. Troubleshooting

No FAM fluorescence signal with positive controls (*CMV QS 1 - 4*):

- The selected detection dye for PCR data analysis does not comply with the protocol.
    - → For data analysis select the detection dye FAM for the analytical CMV PCR and the detection dye VIC/JOE for the *Internal Control* PCR.
- The settings used for the data analysis under *Options* (*Extension Phase Data Extraction*) do not correspond to the settings of the *Data Collection* (for *ABI PRISM 7700 SDS* see **7.6.2.4 Creating the Temperature Profile**, for *ABI PRISM 7900HT SDS* see **7.6.3.4 Creating the Temperature Profile**).
    - → Analyse the PCR run with corrected settings and repeat the data analysis (*Analysis*).
- Incorrect programming of the temperature profile of the *ABI PRISM Sequence Detection System*.
    - → Compare the temperature profile with the protocol (see **7.6 Programming the *ABI PRISM SDS***).
- Incorrect configuration of the PCR reaction.
    - → Check your work steps by means of the pipetting scheme (see **7.5 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 TM 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 (QIAamp DSP Virus Kit) (VIC/JOE fluorescence signal, deviation greater than Ct = 27 ± 3; threshold *ABI PRISM 7000*: 0.1, *ABI PRISM 7700* and *7900HT SDS*: 0.2) and simultaneous absence of a FAM fluorescence signal for the specific CMV PCR:

- 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 the recommended isolation method (see **7.2 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 **7.2 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 **7.2 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 TM 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.

A FAM fluorescence signal of the analytical PCR with the negative controls:
- 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 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.

10. Specifications

10.1 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 TM 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 TM 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 *ABI PRISM 7000*, *7700* and *7900HT Sequence Detection Systems* in combination with the *artus* CMV TM PCR Kit. Testing for all instruments was carried out on three different days on eight replicates. The results were determined by a probit analysis.

Detection limit (p = 0.05)	
ABI PRISM 7000 SDS	0.20 copies/µl
ABI PRISM 7700 SDS	0.20 copies/µl
ABI PRISM 7900HT SDS	0.17 copies/µl

This means that there is a 95 % probability that 0.20 copies/µl (*ABI PRISM 7000 SDS*), 0.20 copies/µl (*ABI PRISM 7700 SDS*) and 0.17 copies/µl (*ABI PRISM 7900HT SDS*) will be detected.

The **analytical sensitivity in consideration of the purification (QIAamp DSP Virus Kit)** of the *artus* CMV TM PCR Kit 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: 70 µl). Each of the eight dilutions was analyzed by means of the *ABI PRISM 7000*, *7700* and *7900HT SDS* with the *artus* CMV TM PCR Kit on three different days on eight replicates. The results were determined by a probit analysis and are shown in the following table:

Detection limit (p = 0.05) in consideration of the purification	
ABI PRISM 7000 SDS	64.2 copies/ml
ABI PRISM 7700 SDS	100.5 copies/ml
ABI PRISM 7900HT SDS	53.5 copies/ml

The graphical illustration is shown in Fig. 29 for the *ABI PRISM 7000 SDS*. The analytical detection limit in consideration of the purification of the *artus* CMV TM PCR Kit is consistently 64.2 copies/ml (p = 0.05). This means that there is a 95 % probability that 64.2 copies/ml will be detected.

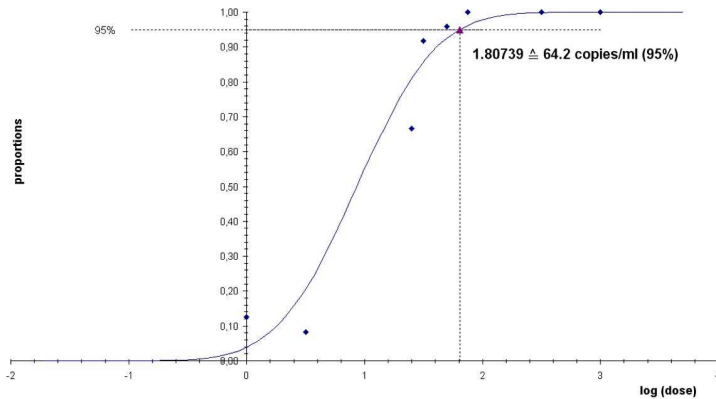**Probit analysis: Cytomegalovirus (*ABI PRISM 7000 SDS*)**

Fig. 29: Analytical sensitivity in consideration of the purification (QIAamp DSP Virus Kit) of the *artus* CMV TM PCR Kit (*ABI PRISM 7000 SDS*).

10.2 Specificity

The specificity of the *artus* CMV TM 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 TM Master*.

To determine the specificity of the *artus* CMV TM 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. No cross-reactivities appeared with mixed infections.

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

Control group	CMV (FAM)	Internal Control (VIC)
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	-	+

10.3 Precision

The precision data of the *artus* CMV TM PCR Kit have been collected by means of the *ABI PRISM 7000 SDS* 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 TM 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). In addition, precision data for quantitative results in copies/µl were determined using the corresponding Ct values (see Table 3). Based on these results, the overall statistical spread of any given sample with the mentioned concentration is 1.06% (Ct) or 12.93% (conc.), for the detection of the *Internal Control* 1.14% (Ct). These values are based on the totality of all single values of the determined variabilities.

Table 2: Precision data on basis of the Ct values.

	Standard deviation	Variance	Coefficient of variation [%]
Intra-assay variability: *CMV LC/RG/TM QS 4*	0.10	0.01	0.33
Intra-assay variability: *Internal Control*	0.12	0.01	0.50
Inter-assay variability: *CMV LC/RG/TM QS 4*	0.21	0.04	0.67
Inter-assay variability: *Internal Control*	0.30	0.09	1.23
Inter-batch variability: *CMV LC/RG/TM QS 4*	0.32	0.10	1.01
Inter-batch variability: *Internal Control*	0.26	0.07	1.05
Total variance: *CMV LC/RG/TM QS 4*	0.33	0.11	1.06
Total variance: *Internal Control*	0.28	0.08	1.14

Table 3: Precision data on basis of the quantitative results (in copies/μl).

	Standard deviation	Variance	Coefficient of variation [%]
Intra-assay variability: *CMV LC/RG/TM QS 4*	0.72	0.52	7.20
Inter-assay variability: *CMV LC/RG/TM QS 4*	1.25	1.57	12.45
Inter-batch variability: *CMV LC/RG/TM QS 4*	1.53	2.33	15.10
Total variance: *CMV LC/RG/TM QS 4*	1.30	1.70	12.93

10.4 Robustness

The verification of the robustness allows the determination of the total failure rate of the *artus* CMV TM 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 **7.2 DNA Isolation**) these samples were analysed with the *artus* CMV TM 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 TM PCR Kit is ≥ 99 %.

11. Product Use Limitations

- For research use only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.
- 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.

12. Warnings and Precautions

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.

13. Quality Control

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

14. References

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

15. Explanation of Symbols
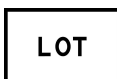
⧖	Use by
LOT	Batch code
▲	Manufacturer
REF	Catalogue number
MAT	Material number
COMP	Components
CONT	Contains
NUM	Number
H B	Handbook
EtOH	Ethanol
GTIN	Global Trade Item Number
$\bigtriangledown^{\Sigma}$ <N>	Contains sufficient for <N> tests
🌡	Temperature limitation

📖	Consult instructions for use
QS	*Quantitation Standard*
IC	*Internal Control*
Mg-Sol	*Magnesium Solution*

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artus CMV TM PCR Kit

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