

March 2015

# artus<sup>®</sup> HSV-1/2 TM PCR Kit Handbook

 24 (catalog no. 4500163)

 96 (catalog no. 4500165)

Quantitative in vitro Diagnostics

For use with the

ABI PRISM<sup>®</sup> 7000, 7700 and 7900HT Sequence Detection Systems

Version 1

**CE**

**IVD**

**REF**

4500163, 4500165

**HB**

1046890



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## artus HSV-1/2 TM PCR Kit

For use with the ABI PRISM 7000 and 7900HT Sequence Detection.

**Attention:** The artus HSV-1/2 TM PCR Kit may neither be used in combination with the GeneAmp® 5700 SDS, neither with the ABI PRISM 7700 SDS nor with the 384 plate format of the ABI PRISM 7900HT SDS.

### 1. Contents

| Labelling and contents |   | Art. No. 4500163<br>24 reactions | Art. No. 4500165<br>96 reactions |
|------------------------|---|----------------------------------|----------------------------------|
| <b>Blue</b>            | HSV TM Master   | 2 x 12 rxns                      | 8 x 12 rxns                      |
| <b>Blue</b>            | HSV TM Master   | 2 x 12 rxns                      | 8 x 12 rxns                      |
| <b>Yellow</b>          | HSV TM Mg-Sol <sup>⌘</sup>                                    | 1 x 1,200 µl                     | 1 x 1,200 µl                     |
| <b>Red</b>             | HSV1 LC/RG/TM QS 1 <sup>⌘</sup><br>1 x 10 <sup>4</sup> cop/µl | 1 x 200 µl                       | 1 x 200 µl                       |
| <b>Red</b>             | HSV1 LC/RG/TM QS 2 <sup>⌘</sup><br>1 x 10 <sup>3</sup> cop/µl | 1 x 200 µl                       | 1 x 200 µl                       |
| <b>Red</b>             | HSV1 LC/RG/TM QS 3 <sup>⌘</sup><br>1 x 10 <sup>2</sup> cop/µl | 1 x 200 µl                       | 1 x 200 µl                       |
| <b>Red</b>             | HSV1 LC/RG/TM QS 4 <sup>⌘</sup><br>1 x 10 <sup>1</sup> cop/µl | 1 x 200 µl                       | 1 x 200 µl                       |
| <b>Red</b>             | HSV2 LC/RG/TM QS 1 <sup>⌘</sup><br>1 x 10 <sup>4</sup> cop/µl | 1 x 200 µl                       | 1 x 200 µl                       |
| <b>Red</b>             | HSV2 LC/RG/TM QS 2 <sup>⌘</sup><br>1 x 10 <sup>3</sup> cop/µl | 1 x 200 µl                       | 1 x 200 µl                       |
| <b>Red</b>             | HSV2 LC/RG/TM QS 3 <sup>⌘</sup><br>1 x 10 <sup>2</sup> cop/µl | 1 x 200 µl                       | 1 x 200 µl                       |
| <b>Red</b>             | HSV2 LC/RG/TM QS 4 <sup>⌘</sup><br>1 x 10 <sup>1</sup> cop/µl | 1 x 200 µl                       | 1 x 200 µl                       |
| <b>Green</b>           | HSV TM IC <sup>⌘</sup>  | 1 x 1,000 µl                     | 2 x 1,000 µl                     |
| <b>White</b>           | Water (PCR grade)   | 1 x 1,000 µl                     | 1 x 1,000 µl                     |

⌘ QS = Quantitation Standard

IC = Internal Control

Mg-Sol = Magnesium Solution

## 2. Storage

The components of the *artus* HSV-1/2 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 8.1 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 8.4 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 8.4 Preparing the PCR
- Compression pad for use with optical adhesive covers (*Optical Cover Compression Pads*, Cat. No. 4 312 639, Applied Biosystems), see 8.4 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* or *7900HT SDS*

**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. Pathogen Information

Herpes simplex virus (HSV) is found in lesion fluids, saliva and vaginal secretions. It is transmitted primarily by direct contact with lesions and via sexual intercourse, as well as perinatally. Lesions on the skin and mucous membranes of the mouth and genitals characterize most HSV positive cases. HSV infection can be either primary (> 90 % of these cases are asymptomatic) or recurrent (secondary). Primary infection with HSV-1 can lead to, among others, gingivostomatitis, eczema herpeticum, keratoconjunctivitis and encephalitis; primary HSV-2 infection occurs as, among others, vulvovaginitis, meningitis and generalized herpes in newborns. The primary symptoms of a secondary infection are skin lesions in the nose, mouth and genital regions. Even more severe are the recurrent forms of keratoconjunctivitis and meningitis.

## 6. Principle of Real-Time PCR

Pathogen diagnosis by the polymerase chain reaction (PCR) is based on the amplification of specific regions of the pathogen genome. In real-time PCR the amplified product is detected via fluorescent dyes. These are usually linked to oligonucleotide probes which bind specifically to the amplified product. Monitoring the fluorescence intensities during the PCR run (i.e. in real-time) allows the detection and quantitation of the accumulating product without having to re-open the reaction tubes after the PCR run (Mackay, 2004).

## 7. Product Description

The *artus* HSV-1/2 TM PCR Kit constitutes a ready-to-use system for the detection and differentiation of herpes simplex virus-1 and -2 DNA using polymerase chain reaction (PCR) in the *ABI PRISM 7000* and *7900HT Sequence Detection System*. The *HSV TM Master* contains reagents and enzymes for the specific amplification of a 148 bp region of the herpes simplex virus genome. The amplicon is detected by measuring the FAM fluorescence (HSV-1) and NED

fluorescence (HSV-2) in the *ABI PRISM SDS*. In addition, the *artus HSV-1/2 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 fluorescence. The detection limit of the analytical HSV PCR (see 11.1 Analytical Sensitivity) is not reduced. External positive controls (*HSV1 LC/RG/TM QS 1 – 4* & *HSV2 LC/RG/TM QS 1 – 4*) are supplied which allow the determination of the pathogen load. For further information, please refer to section 8.3 Quantitation.

## 8. Protocol

### 8.1 DNA Isolation

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

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

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

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

- The use of carrier RNA is critical for the extraction efficiency and, consequently, for DNA/RNA yield. If the selected isolation kit does not contain carrier RNA, please note that the addition of carrier (RNA Homopolymer Poly(A), Amersham Biosciences, Cat. No. 27-4110-01) is strongly recommended for the extraction of nucleic acids from cell free body fluids and material low in DNA/RNA content (e.g. CSF). Please proceed as follows in these cases:
  - a) Resuspend the lyophilised carrier RNA using the elution buffer (do not use lysis buffer) of the extraction kit (e.g. AE buffer of the QIAamp DNA Mini Kit) and prepare a dilution with a concentration of 1 µg/µl. Divide this carrier RNA solution in a number of aliquots adequate to your needs and store them at -20°C. Avoid repeated thawing (> 2 x) of a carrier RNA aliquot.
  - b) Use 1 µg carrier RNA per 100 µl lysis buffer. For instance, if the extraction protocol suggests 200 µl lysis buffer, please add 2 µl carrier RNA (1 µg/µl) directly into the lysis buffer. Before beginning of each extraction, a mixture of lysis buffer and carrier RNA (and *Internal Control*, where applicable, see 8.2 *Internal Control*) should be prepared freshly according to the following pipetting scheme:

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

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

- The use of **carrier RNA** is critical for the extraction efficiency and, consequently, for DNA/RNA yield. To increase the stability of the carrier RNA provided with the QIAamp UltraSens Virus Kit, we recommend the following procedure deviant from the user manual of the extraction kit:
  - a) Resuspend the lyophilised carrier RNA prior to first use of the extraction kit in 310  $\mu\text{l}$  of the elution buffer provided with the kit (final concentration 1  $\mu\text{g}/\mu\text{l}$ , do not use lysis buffer). Portion this carrier RNA solution into a number of aliquots adequate to your needs and store them at  $-20^{\circ}\text{C}$ . Avoid repeated thawing ( $> 2 \times$ ) of a carrier RNA aliquot.
  - b) Before the beginning of each extraction, a mixture of lysis buffer and carrier RNA (and *Internal Control*, where applicable, see 8.2 *Internal Control*) should be prepared freshly according to the following pipetting scheme:

| <b>Number of samples</b>                   | <b>1</b>                              | <b>12</b>                                |
|--|---------------------------------------|--|
| Lysis buffer AC                            | 800 $\mu\text{l}$                     | 9,600 $\mu\text{l}$                      |
| Carrier RNA (1 $\mu\text{g}/\mu\text{l}$ ) | 5.6 $\mu\text{l}$                     | 67.2 $\mu\text{l}$                       |
| <b>Total Volume</b>                        | <b>805.6 <math>\mu\text{l}</math></b> | <b>9,667.2 <math>\mu\text{l}</math></b>  |
| <b>Volume per extraction</b>               | <b>800 <math>\mu\text{l}</math></b>   | <b>each 800 <math>\mu\text{l}</math></b> |

- c) Please use the freshly prepared mixture of lysis buffer and carrier RNA instantly for extraction. Storage of the mixture is not possible.
- It is recommended to elute the DNA in 50  $\mu\text{l}$  elution buffer to get the highest sensitivity of the *artus* HSV-1/2 TM PCR Kit.
  - The **QIAamp UltraSens Virus Kit** allows a sample concentration. If you use sample material other than serum or plasma, please add at least 50 % (v/v) of negative human plasma to the sample.
  - When using isolation protocols with **ethanol**-containing washing buffers, please carry out an additional centrifugation step (three minutes, 13,000 rpm) before the elution to remove any remaining ethanol. This prevents possible inhibition of PCR.
  - The *artus* HSV-1/2 TM PCR Kit should not be used with **phenol**-based isolation methods.

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

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

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

## 8.2 *Internal Control*

An *Internal Control (HSV TM IC)* is supplied. This allows the user **both to control the DNA isolation procedure and to check for possible PCR inhibition** (see Fig. 1). Using the **EZ1 DSP Virus Kit** for extraction, the *Internal Control* has to be added following the instructions in the *EZ1 DSP Virus Kit Handbook*. Using the **QIAamp UltraSens Virus Kit** or the **QIAamp DNA Mini Kit**, add the *Internal Control* to the isolation at a ratio of 0.1  $\mu\text{l}$  per 1  $\mu\text{l}$  elution volume. For example, using the QIAamp DNA Mini Kit, the DNA is eluted in 50  $\mu\text{l}$  AE buffer. Hence, 5  $\mu\text{l}$  of the *Internal Control* should be added initially. The quantity of *Internal Control* used depends **only** on the elution volume. The *Internal Control* and carrier RNA (see 8.1 DNA Isolation) should be added only

- to the mixture of lysis buffer and sample material or
- directly to the lysis buffer.

The *Internal Control* must not be added to the sample material directly. If added to the lysis buffer please note that the mixture of *Internal Control* and lysis buffer/carrier RNA has to be prepared freshly and used instantly (storage of the mixture at room temperature or in the fridge for only a few hours may lead to *Internal Control* failure and a reduced extraction efficiency). Please do not add the *Internal Control* and the carrier RNA to the sample material directly.

The *Internal Control* can optionally be used **exclusively to check for possible PCR inhibition** (see Fig. 2). For this application, add 2  $\mu\text{l}$  of the *Internal Control* and 10  $\mu\text{l}$  *HSV TM Mg-Sol* per reaction directly to 20  $\mu\text{l}$  *HSV TM Master*. For each PCR reaction use 30  $\mu\text{l}$  of the Master Mix produced as described above\* and add 20  $\mu\text{l}$  of the purified sample. If you are preparing a PCR run for several samples please increase the volume of the *HSV TM Master*, the *HSV TM Mg-Sol* and the *Internal Control* according to the number of samples (see 8.4 Preparing the PCR).

## 8.3 Quantitation

The enclosed *Quantitation Standards (HSV1 LC/RG/TM QS 1 – 4 & HSV2 LC/RG/TM QS 1 – 4)* are treated as previously purified samples and the same volume is used (20  $\mu\text{l}$ ). To generate a standard curve on an *ABI PRISM Sequence Detection System*, all four *Quantitation Standards* of HSV-1 as well as HSV-2 should be used and defined as standards with specification of the corresponding concentrations (see 8.5 Programming the *ABI PRISM SDS*). The

\* The volume increase caused by adding the *Internal Control* is neglected when preparing the PCR assay. The sensitivity of the detection system is not impaired.

import of standard curves from previous runs is not possible with the *ABI PRISM 7000* and *7900HT SDS* software.

**Attention:** The *Quantitation Standards* are defined as copies/ $\mu$ l. 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 *ABI PRISM 7000 SDS* is provided at [www.qiagen.com/Products/ByLabFocus/MDX](http://www.qiagen.com/Products/ByLabFocus/MDX) (**Technical Note for quantitation on the *ABI PRISM 7000 SDS***).

## 8.4 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              |

When preparing the PCR reaction, please make sure that at least one *Quantitation Standard* per PCR run as well as one negative control (*Water, PCR grade*) are performed in parallel. To generate a standard curve, use all supplied *Quantitation Standards (HSV1 LC/RG/TM QS 1 – 4 & HSV2 LC/RG/TM QS 1 – 4)* for each PCR run. Before each use, all reagents need to be thawed completely, mixed (by repeated up and down pipetting or by quick vortexing) and centrifuged briefly.

If you want to use the *Internal Control* to **monitor the DNA isolation procedure and to check for possible PCR inhibition**, it has already been

\* 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.

added to the isolation (see 8.2 *Internal Control*). In this case, please use the following pipetting scheme (for a schematic overview see Fig. 1):

|                                     |                      | <b>Number of samples</b>    |                                  |
|-------------------------------------|----------------------|-----------------------------|----------------------------------|
|                                     |                      | <b>1</b>                    | <b>12</b>                        |
| <b>1. Preparation of Master Mix</b> | <i>HSV TM Master</i> | 20 $\mu$ l                  | 240 $\mu$ l                      |
|                                     | <i>HSV TM Mg-Sol</i> | 10 $\mu$ l                  | 120 $\mu$ l                      |
|                                     | <i>HSV TM IC</i>     | 0 $\mu$ l                   | 0 $\mu$ l                        |
|                                     | <b>Total Volume</b>  | <b>30 <math>\mu</math>l</b> | <b>360 <math>\mu</math>l</b>     |
| <b>2. Preparation of PCR assay</b>  | Master Mix           | 30 $\mu$ l                  | 30 $\mu$ l each                  |
|                                     | Sample               | 20 $\mu$ l                  | 20 $\mu$ l each                  |
|                                     | <b>Total Volume</b>  | <b>50 <math>\mu</math>l</b> | <b>50 <math>\mu</math>l each</b> |

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

|                                     |                      | <b>Number of samples</b>     |                                  |
|-------------------------------------|----------------------|------------------------------|----------------------------------|
|                                     |                      | <b>1</b>                     | <b>12</b>                        |
| <b>1. Preparation of Master Mix</b> | <i>HSV TM Master</i> | 20 $\mu$ l                   | 240 $\mu$ l                      |
|                                     | <i>HSV TM Mg-Sol</i> | 10 $\mu$ l                   | 120 $\mu$ l                      |
|                                     | <i>HSV TM IC</i>     | 2 $\mu$ l                    | 24 $\mu$ l                       |
|                                     | <b>Total Volume</b>  | <b>32 <math>\mu</math>l*</b> | <b>384 <math>\mu</math>l*</b>    |
| <b>2. Preparation of PCR assay</b>  | Master Mix           | 30 $\mu$ l*                  | 30 $\mu$ l* each                 |
|                                     | Sample               | 20 $\mu$ l                   | 20 $\mu$ l each                  |
|                                     | <b>Total Volume</b>  | <b>50 <math>\mu</math>l</b>  | <b>50 <math>\mu</math>l each</b> |

Pipette 30  $\mu$ l of the Master Mix into each required reaction tube or well of the 96-well reaction plate. Subsequently add 20  $\mu$ l 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

\* 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.

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 8.5 Programming the *ABI PRISM SDS*) and subsequently transfer them into the instrument.

**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* 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 **lower** 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

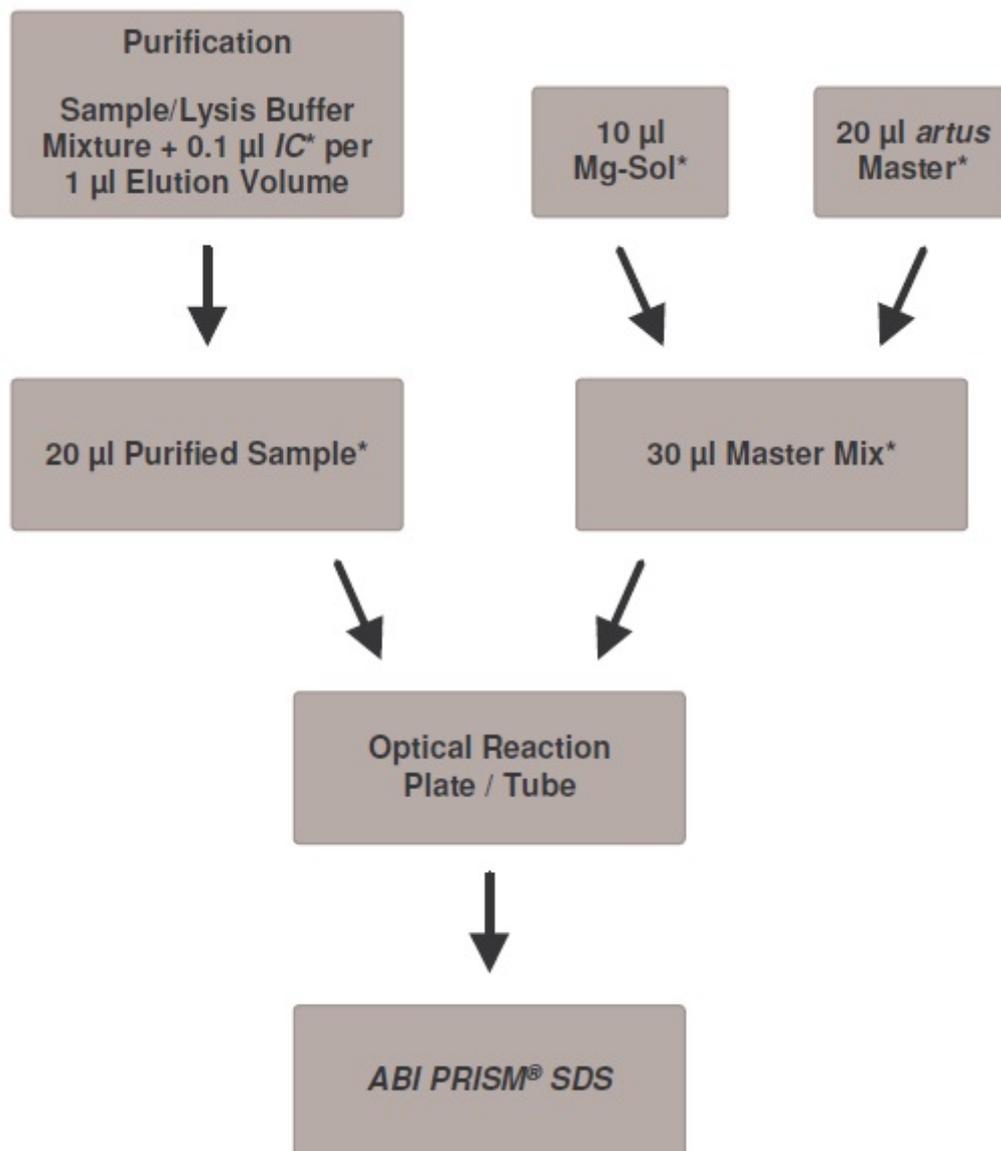


Fig. 1: Schematic workflow for the control of both the purification procedure and PCR inhibition.

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

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

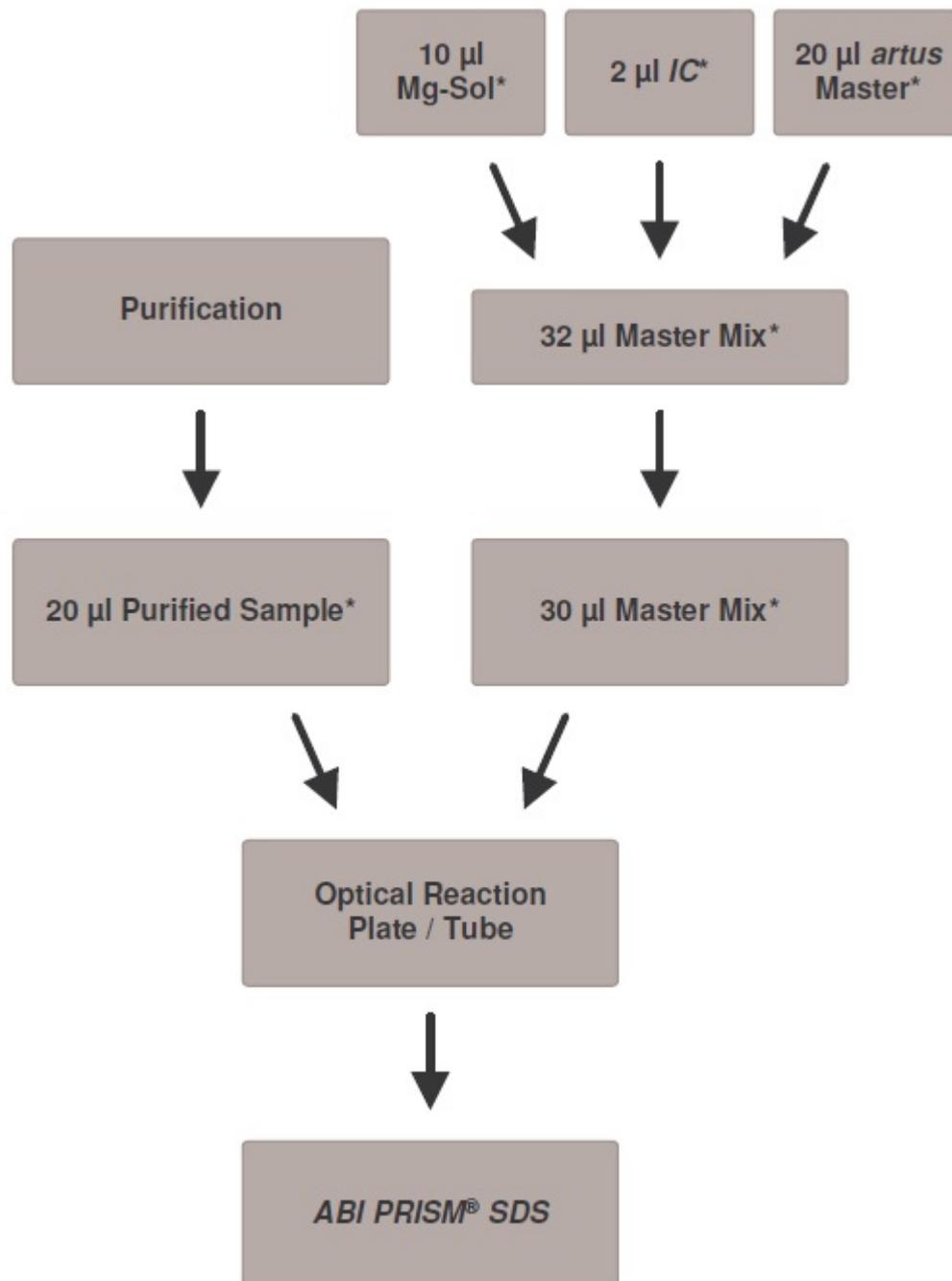


Fig. 2: Schematic workflow for the control of PCR inhibition.

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

## 8.5 Programming the **ABI PRISM SDS**

The software of the *ABI PRISM 7000* 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.

### 8.5.1 Programming the **ABI PRISM 7000 SDS**

For the detection of HSV DNA, create a profile on your *ABI PRISM 7000 SDS* according to the following six steps (8.5.1.1 – 8.5.1.6). 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.

#### 8.5.1.1 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 8.5.1.5 Saving the PCR Run).

Confirm your pre-settings (OK).

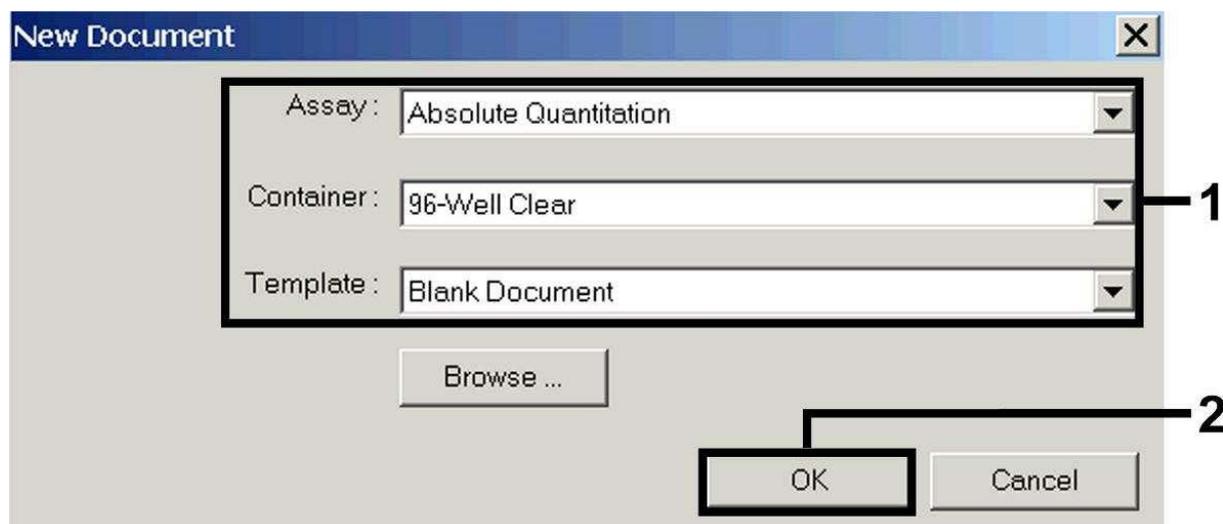


Fig. 3: Pre-settings for creating a new PCR run (*New Document*).

#### 8.5.1.2 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 HSV DNA as well as the *Internal Control* by means of the *artus HSV-1/2 TM PCR Kit*, the reporters/quenchers listed in the following table are to be defined:

| Detection                           | Reporter | Quencher |
|-------------------------------------|----------|----------|
| HSV-1 DNA                           | FAM      | None     |
| HSV-2 DNA                           | NED      | None     |
| <i>Internal Control (HSV TM IC)</i> | VIC      | none     |

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

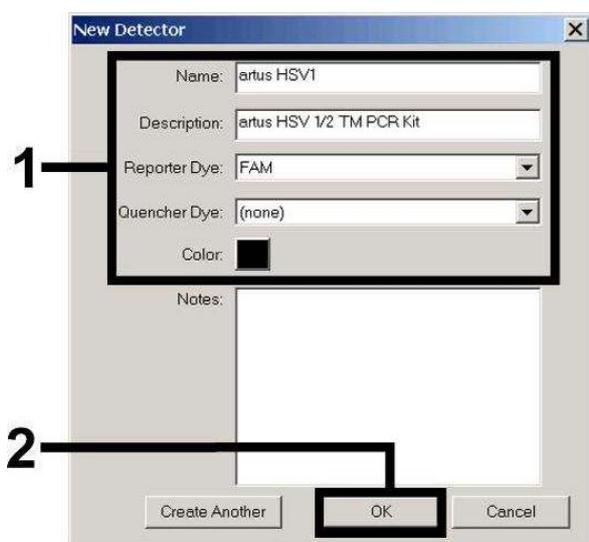


Fig. 4: Creating the HSV-1 specific detector (*Detector Manager*).

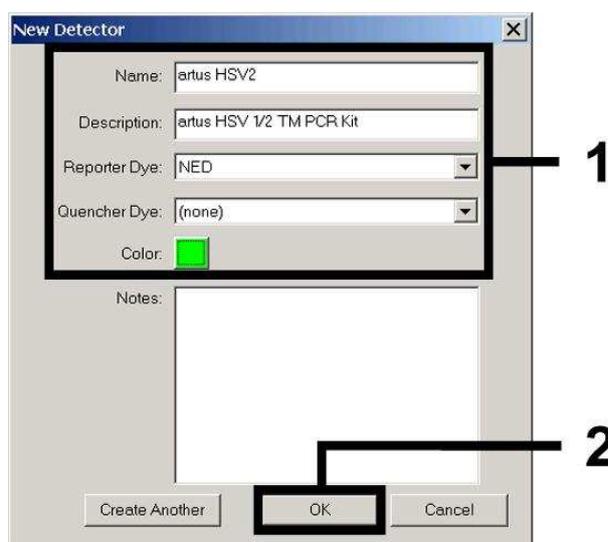


Fig. 5: Creating the HSV-2 specific detector (*Detector Manager*).

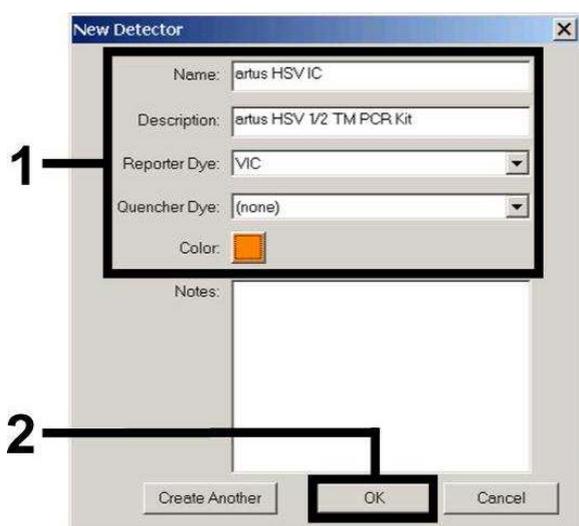


Fig. 6: Creating the IC specific detector (*Detector Manager*) for HSV-1/HSV-2.

For the detection of HSV-1 DNA or HSV-2 DNA define the reporter/quencher combination **FAM/none** or **NED/none** in the new window. For the detection of the *Internal Control*, select the combination **VIC/none** (as shown in Fig. 4 – Fig. 6). 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. 7). Close the window (*Done*).

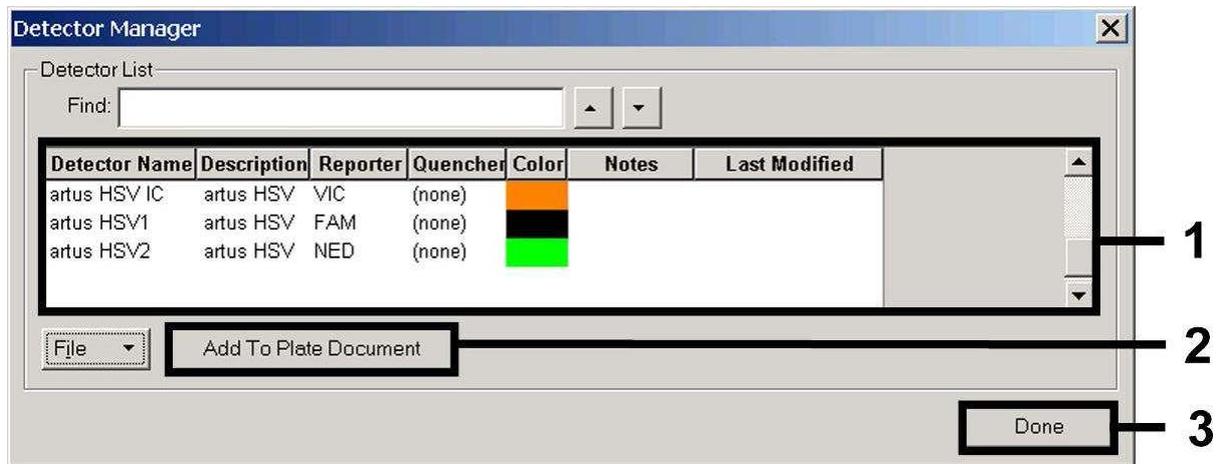


Fig. 7: Selecting the detectors (*Detector Manager*).

### 8.5.1.3 Assigning the Necessary Information to the Plate Positions

Open *Well Inspector* from the View menu to find the detectors selected under 8.5.1.2 (see Fig. 8 – Fig. 9).

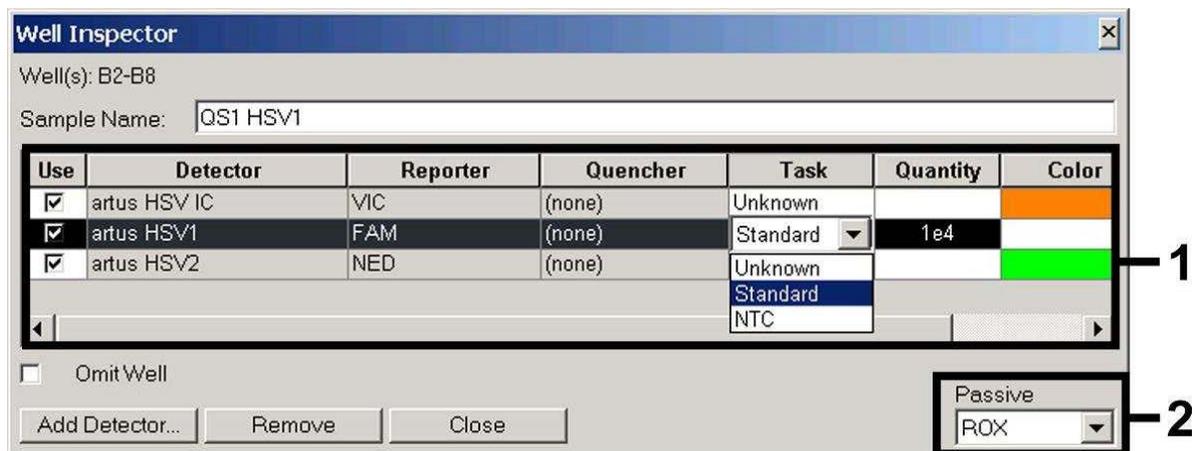


Fig. 8: Assigning the necessary information (HSV-1) to the plate positions (*Well Inspector*).

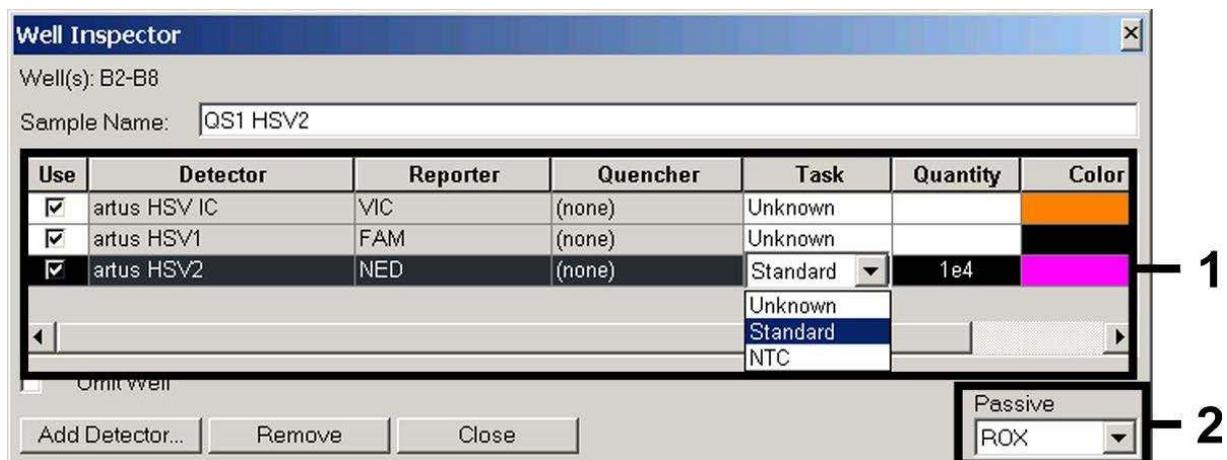


Fig. 9: Assigning the necessary information (HSV-2) to the plate positions (*Well Inspector*).

Mark the plate positions reserved for the detection of HSV DNA. Assign the selected detectors to these positions by activating the *Use* option of all three 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:

| <b>Sample Type</b>   | <b>Function (Task)</b> | <b>Concentration (Quantity)</b> | <b>Reporter HSV-1</b> | <b>Reporter HSV-2</b> | <b>Quencher</b> |
|----------------------|------------------------|---------------------------------|-----------------------|-----------------------|-----------------|
| Sample               | Unknown                | –                               | FAM                   | NED                   | none            |
| Non-template control | NTC                    | –                               | FAM                   | NED                   | none            |
| Standard             | Standard               | see 1. Contents                 | FAM                   | NED                   | none            |

To generate a standard curve, use all supplied *Quantitation Standards (HSV1 LC/RG/TM QS 1 – 4 & HSV2 LC/RG/TM 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 HSV-1/2 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 *HSV 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).

#### **8.5.1.4 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 HSV DNA according to Fig. 10. 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*).

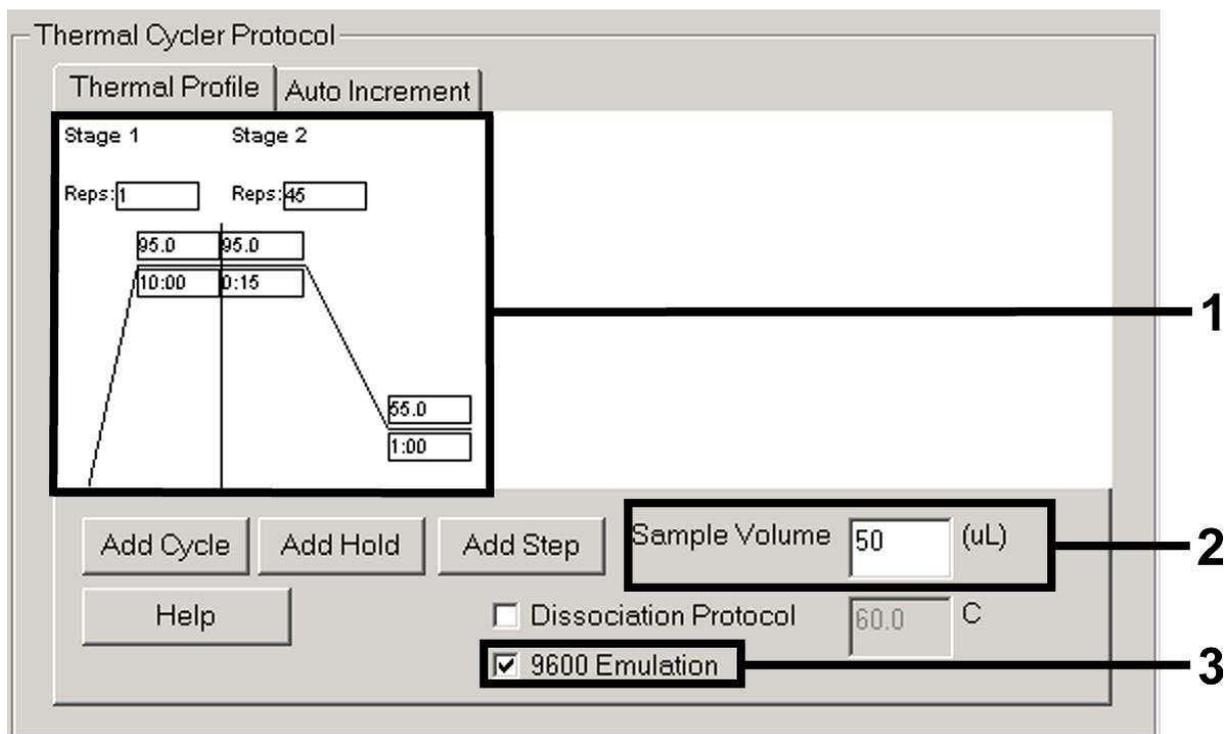


Fig. 10: Creating the temperature profile.

### 8.5.1.5 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.

### 8.5.1.6 Starting the PCR Run

Start the PCR run by selecting the option *Start*

## 8.5.2 Programming the ABI PRISM 7900HT SDS

For the detection of HSV DNA, create a profile on your *ABI PRISM 7900HT SDS* according to the following six steps (8.5.2.1 – 8.5.2.6). 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.

### 8.5.2.1 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. 11). A backup template (*ABI PRISM SDS Template Document (\*.sdt)*) is available from

the *Template* list or by selection using the *Browse* function (see 8.5.2.5 Saving the PCR Run). Confirm the pre-settings (*OK*).

**Attention:** The *artus HSV-1/2 TM PCR Kit* may not be used in combination with the 384 plate format of the *ABI PRISM 7900HT SDS*.

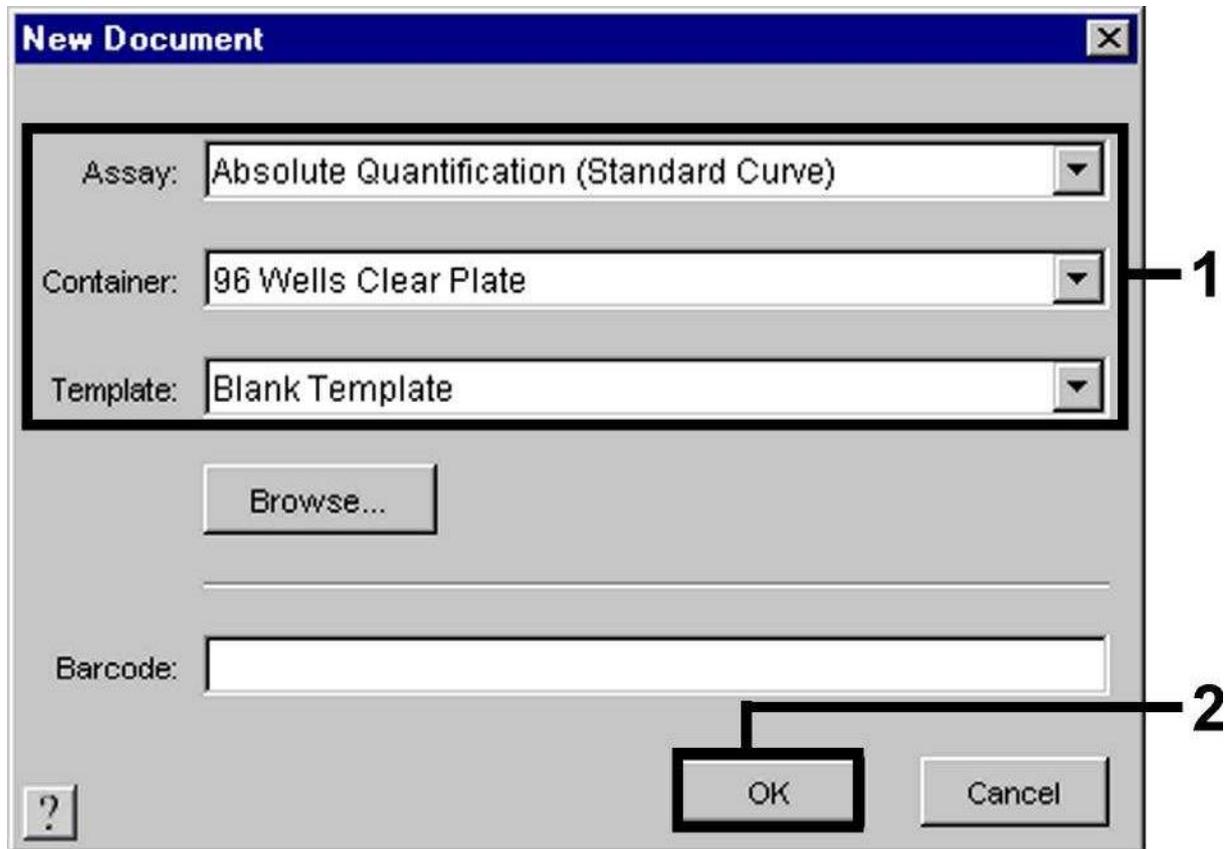


Fig. 11: Pre-settings for creating a new PCR run (*New Document*).

### 8.5.2.2 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 HSV DNA as well as the *Internal Control* by means of the *artus HSV-1/2 TM PCR Kit*, the reporters/quenchers listed in the following table are to be defined:

| Detection                           | Reporter | Quencher        |
|-------------------------------------|----------|-----------------|
| HSV-1 DNA                           | FAM      | Non Fluorescent |
| HSV-2 DNA                           | NED      | Non Fluorescent |
| <i>Internal Control (HSV TM IC)</i> | VIC      | Non Fluorescent |

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

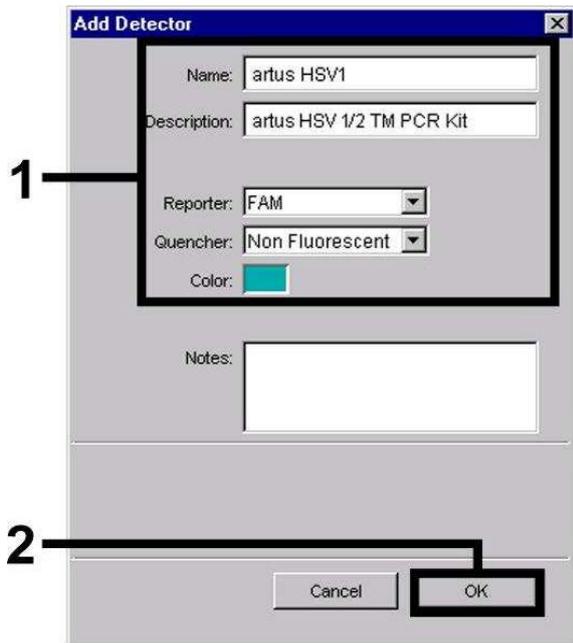


Fig. 12: Creating the HSV-1 specific detector (*Detector Manager*).

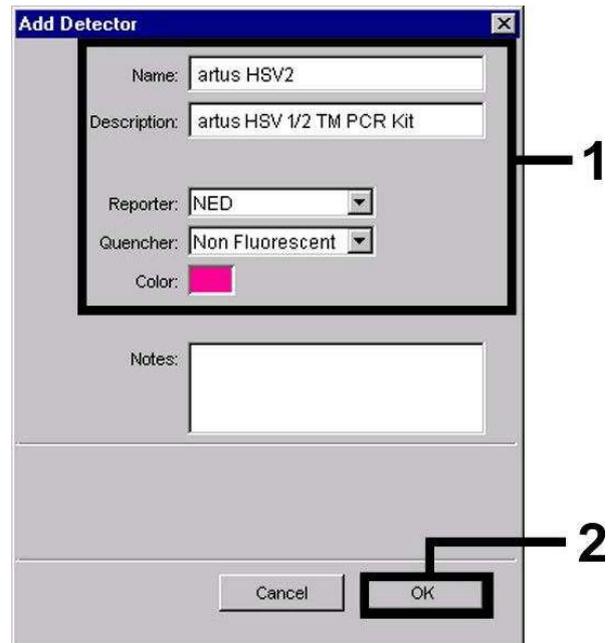


Fig. 13: Creating the HSV-2 specific detector (*Detector Manager*).

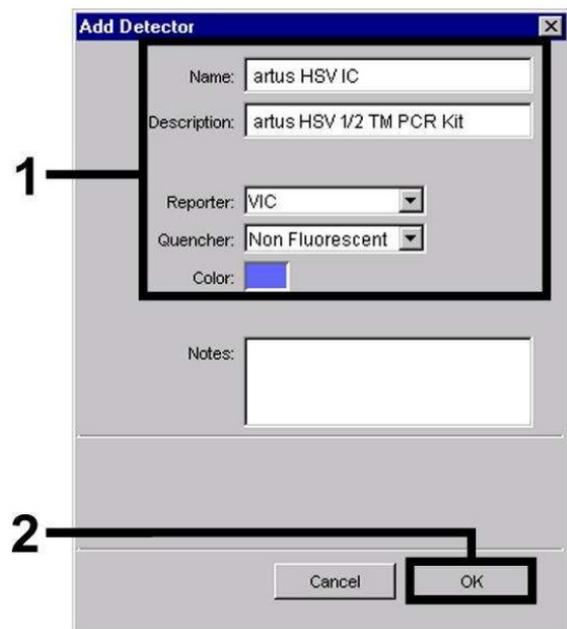


Fig. 14: Creating the *IC* specific detector (*Detector Manager*) for HSV-1/HSV-2.

For the detection of HSV-1 DNA and HSV-2 DNA define the reporter/quencher combination **FAM/Non Fluorescent** or **NED/Non Fluorescent** in the new window. For the detection of the *Internal Control*, select the combination **VIC/Non Fluorescent** (as shown in Fig. 12 – Fig. 14). 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. 15). Close the window (*Done*).

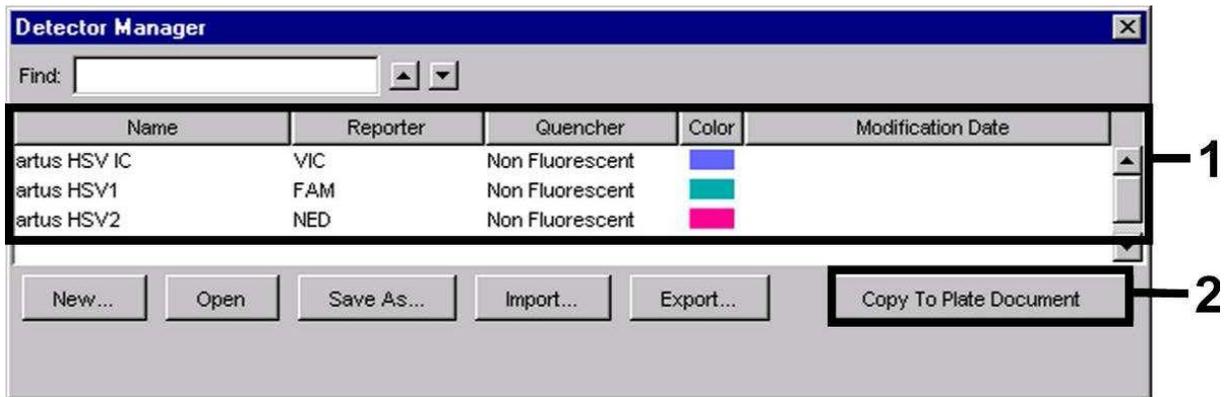


Fig. 15: Selecting the detectors (*Detector Manager*).

### 8.5.2.3 Assigning the Necessary Information to the Plate Positions

After having closed the *Detector Manager* (*Done*) the detectors selected under 8.5.2.2 are found on the *Setup* level listed in a table (see Fig. 16 – Fig. 17).

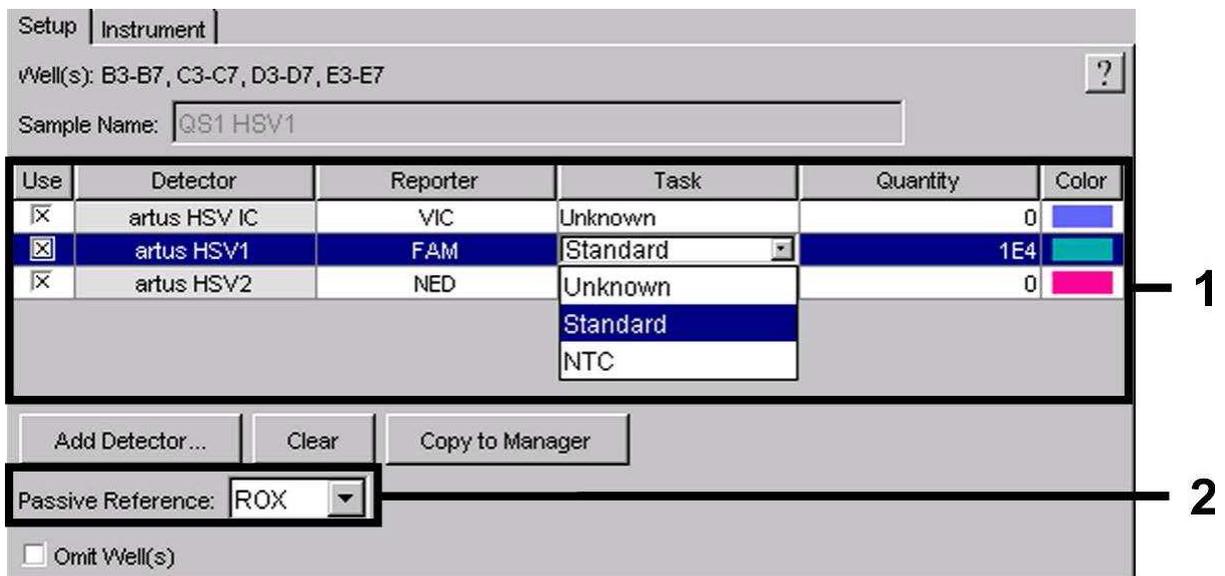


Fig. 16: Assigning the necessary information (HSV-1) to the plate positions.

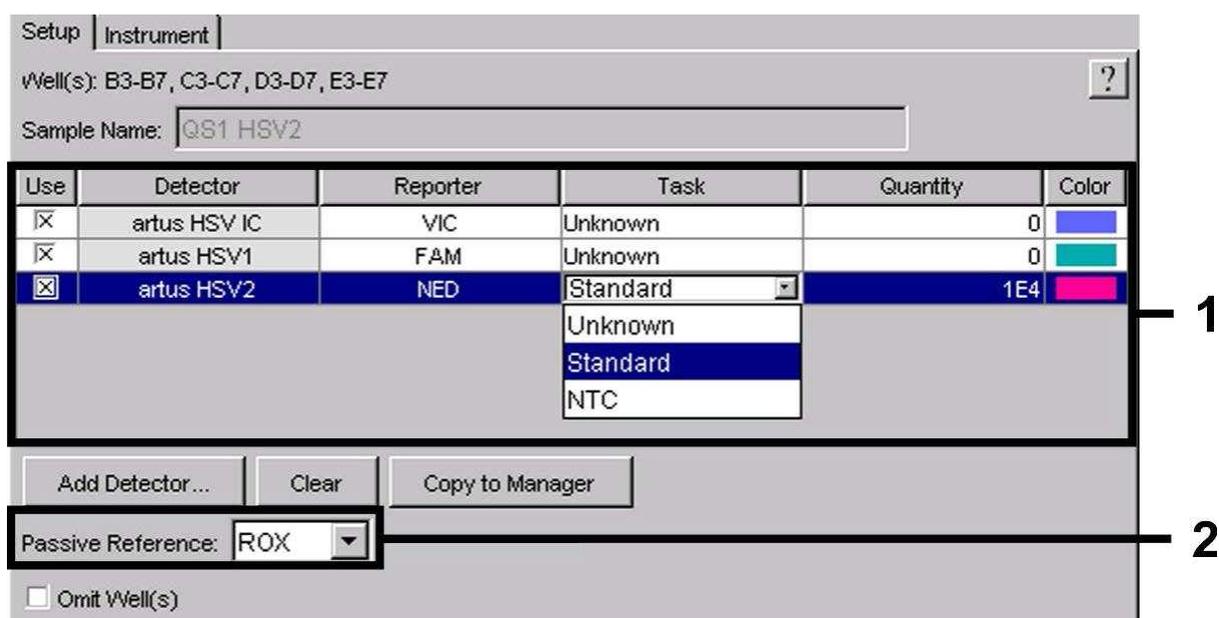


Fig. 17: Assigning the necessary information (HSV-2) to the plate positions.

Mark the plate positions reserved for the detection of HSV DNA. Assign the selected detectors to these positions by activating the *Use* option of all three 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 HSV-1 | Reporter HSV-2 | Quencher        |
|----------------------|-----------------|--------------------------|----------------|----------------|-----------------|
| Sample               | Unknown         | –                        | FAM            | NED            | Non Fluorescent |
| Non-template control | NTC             | –                        | FAM            | NED            | Non Fluorescent |
| Standard             | Standard        | see 1. Contents          | FAM            | NED            | Non Fluorescent |

To generate a standard curve, use all supplied *Quantitation Standards (HSV1 LC/RG/TM QS 1 – 4 & HSV2 LC/RG/TM 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 HSV-1/2 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 *HSV 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).

#### 8.5.2.4 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 HSV DNA according to Fig. 18. Make sure that the reaction volume is set to 50  $\mu\text{l}$ . 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).

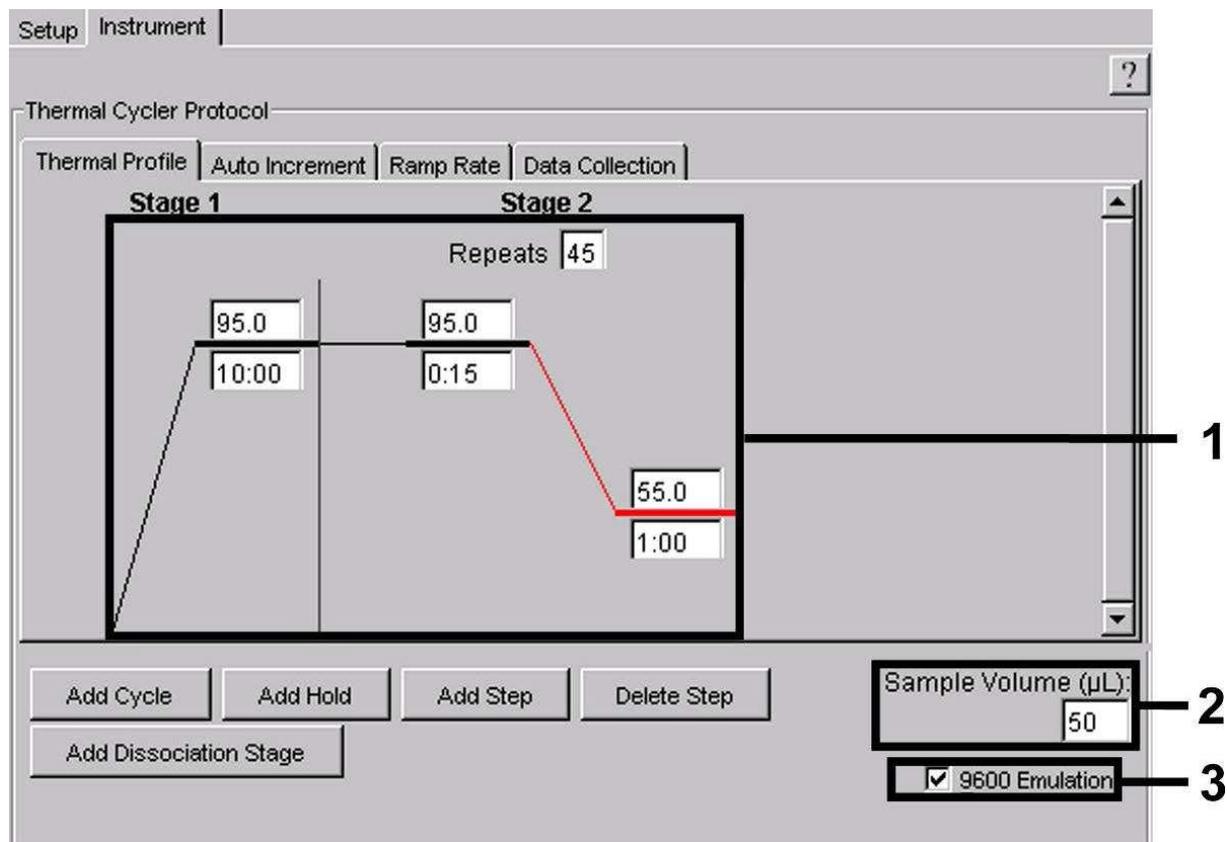


Fig. 18: Creating the temperature profile.

In addition, the *Instrument* level contains the option *Data Collection*. By selecting this option the window depicted in Fig. 19 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-Extension* 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.

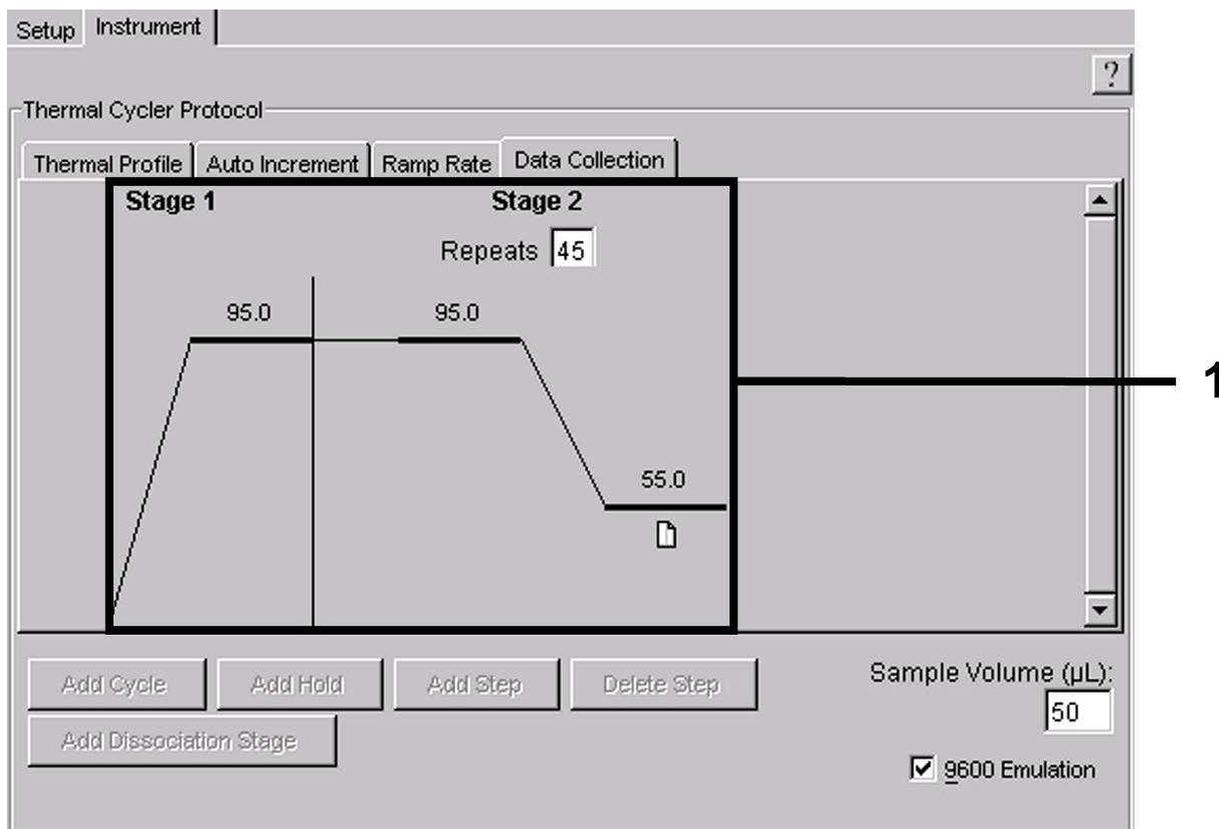


Fig. 19: Data collection.

### 8.5.2.5 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.

### 8.5.2.6 Starting the PCR Run

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

## 9. 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*™ 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.

The following results are possible:

1. A FAM fluorescence signal for HSV-1 or NED fluorescence signal for HSV-2 are detected.

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

In this case, the detection of a VIC fluorescence signal (*Internal Control*) is dispensable, since high initial concentrations of HSV DNA (positive FAM fluorescence signal for HSV-1 and NED fluorescence signal for HSV-2) can lead to a reduced or absent fluorescence signal of the *Internal Control* (competition).

- Neither a FAM fluorescence signal for HSV-1 nor a NED fluorescence signal for HSV-2 are detected. At the same time, a VIC fluorescence signal from the *Internal Control* appears.

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

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

- Neither a FAM fluorescence signal for HSV-1 nor a NED fluorescence signal for HSV-2 nor a VIC fluorescence signal are detected.

**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. 20 – 23 (*ABI PRISM 7000 SDS*) and 24 – 27 (*ABI PRISM 7900HT SDS*).

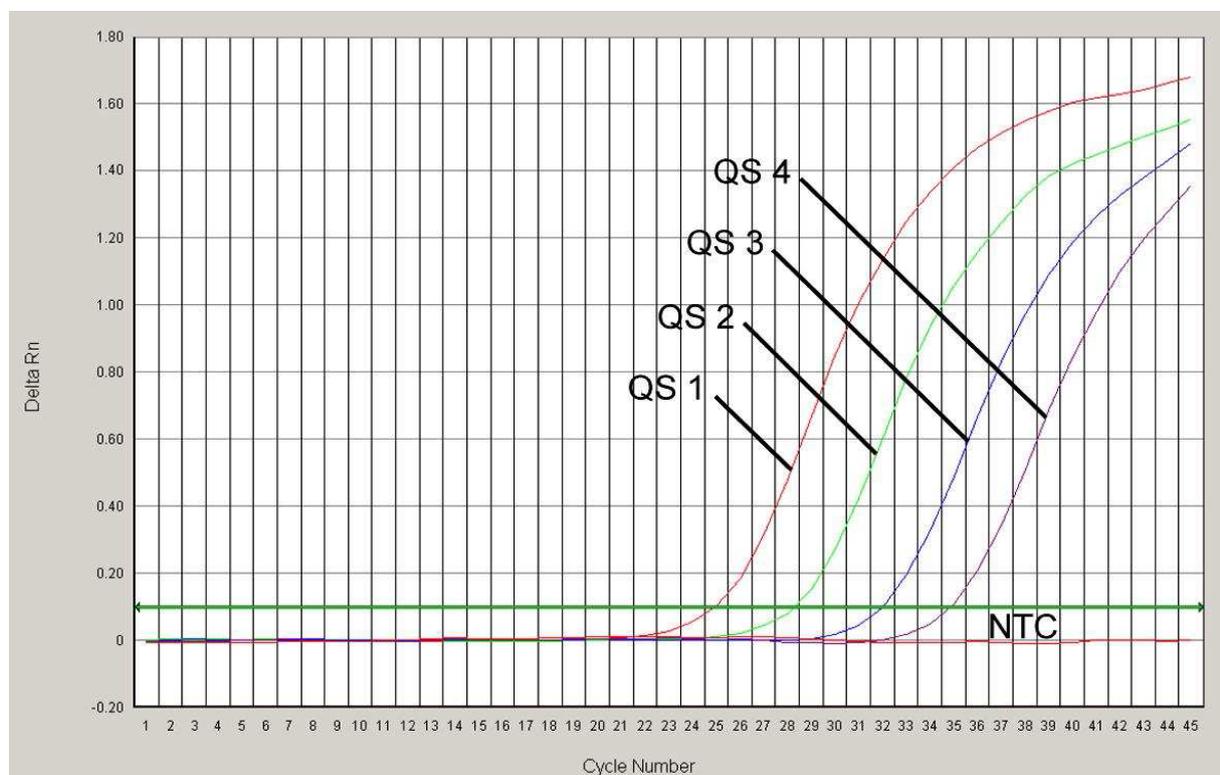


Fig. 20: Detection of the *Quantitation Standards (HSV1 LC/RG/TM QS 1 – 4)* by measurement of the FAM fluorescence signal (***ABI PRISM 7000 SDS***). NTC: non-template control (negative control).

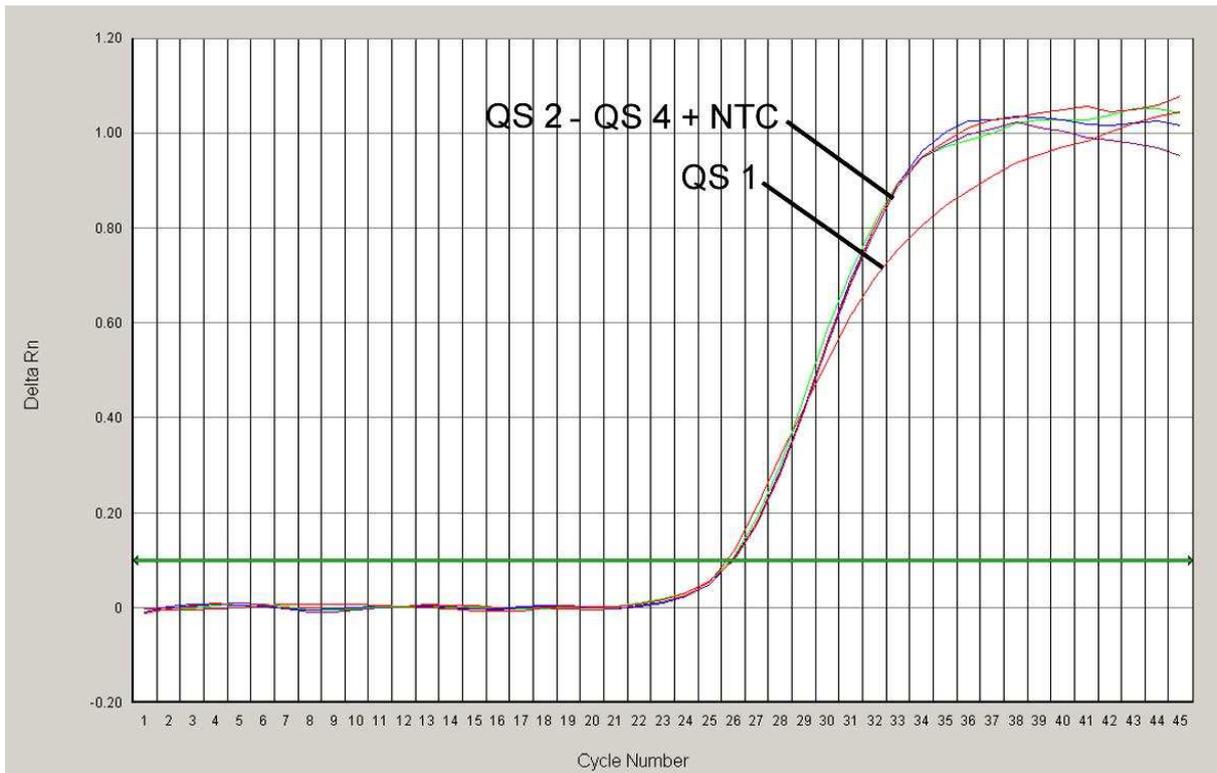


Fig. 21: Detection of the *Internal Control (IC)* by measurement of the VIC fluorescence signal (**ABI PRISM 7000 SDS**) with simultaneous amplification of the *Quantitation Standards (HSV1 LC/RG/TM QS 1 – 4)*. NTC: non-template control (negative control).

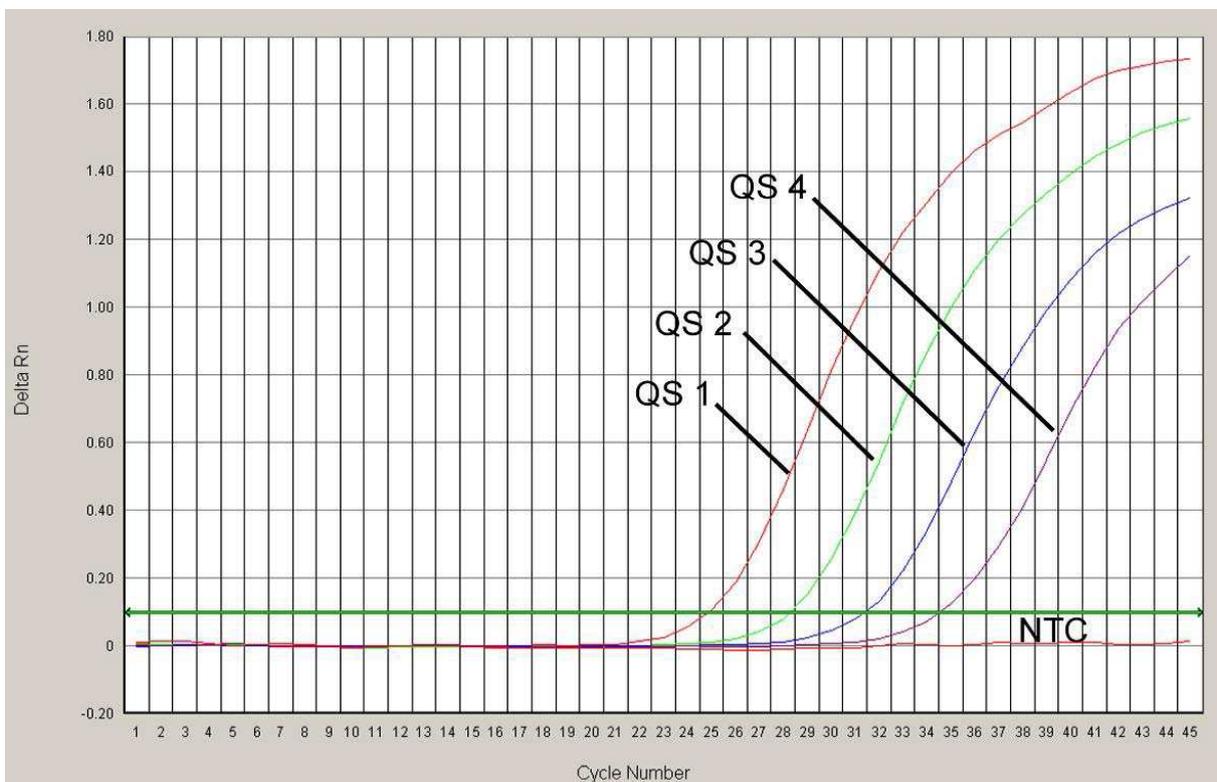


Fig. 22: Detection of the *Quantitation Standards (HSV2 LC/RG/TM QS 1 – 4)* by measurement of the NED fluorescence signal (**ABI PRISM 7000 SDS**). NTC: non-template control (negative control).

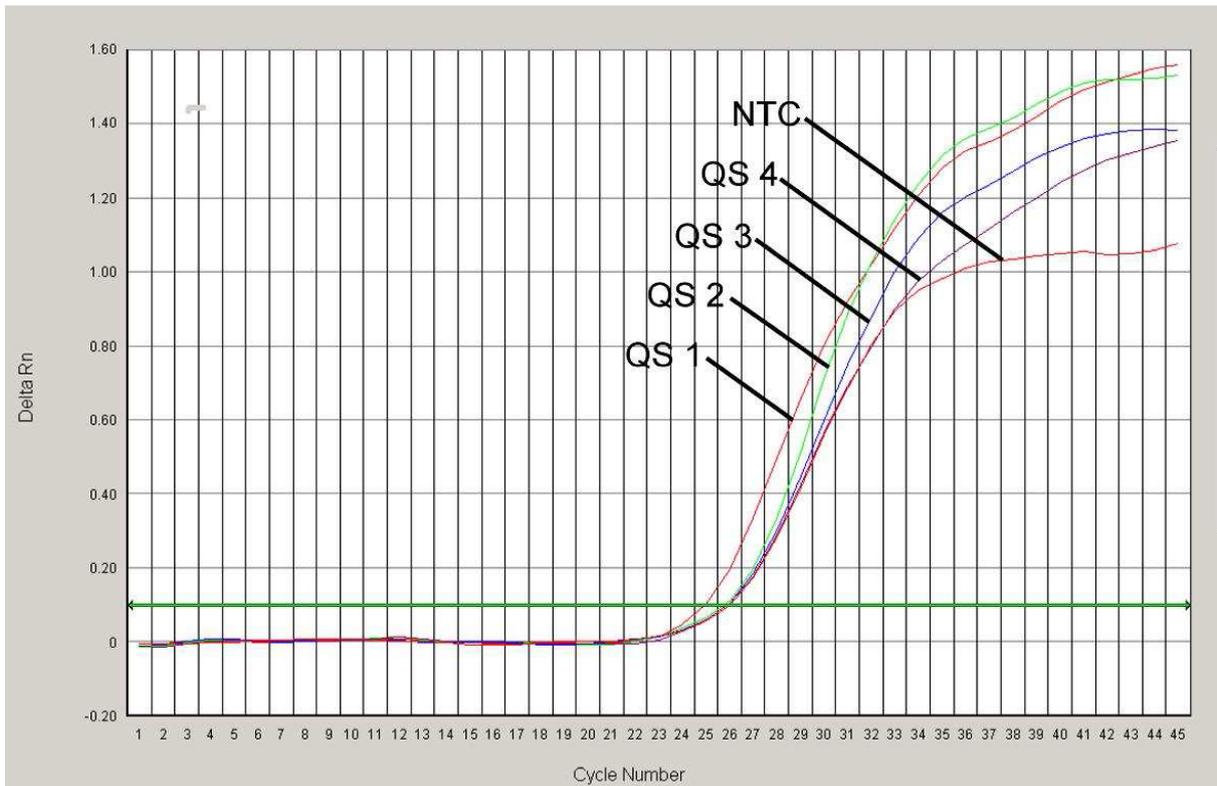


Fig. 23: Detection of the *Internal Control (IC)* by measurement of the VIC fluorescence signal (**ABI PRISM 7000 SDS**) with simultaneous amplification of the *Quantitation Standards (HSV2 LC/RG/TM QS 1 – 4)*. NTC: non-template control (negative control).

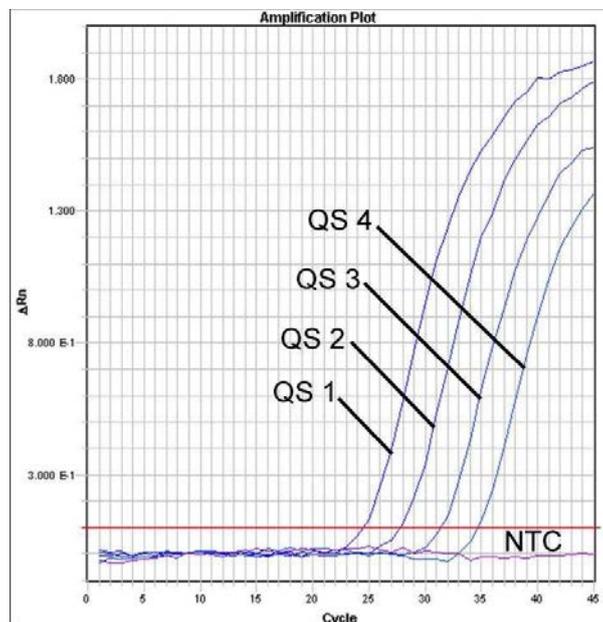


Fig. 24: Detection of the *Quantitation Standards (HSV1 LC/RG/TM QS 1 – 4)* by measurement of the FAM fluorescence signal (**ABI PRISM 7900HT SDS**). NTC: non-template control (negative control).

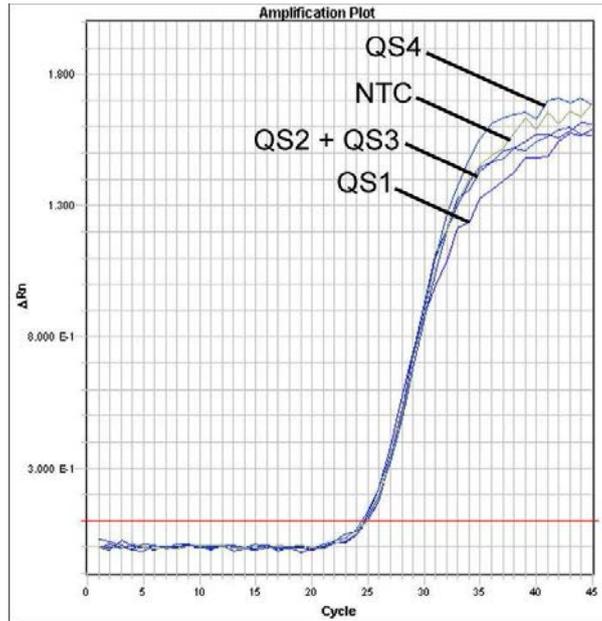


Fig. 25: Detection of the *Internal Control (IC)* by measurement of the VIC fluorescence signal (**ABI PRISM 7900HT SDS**) with simultaneous amplification of the *Quantitation Standards (HSV1 LC/RG/TM QS 1 – 4)*. NTC: non-template control (negative control).

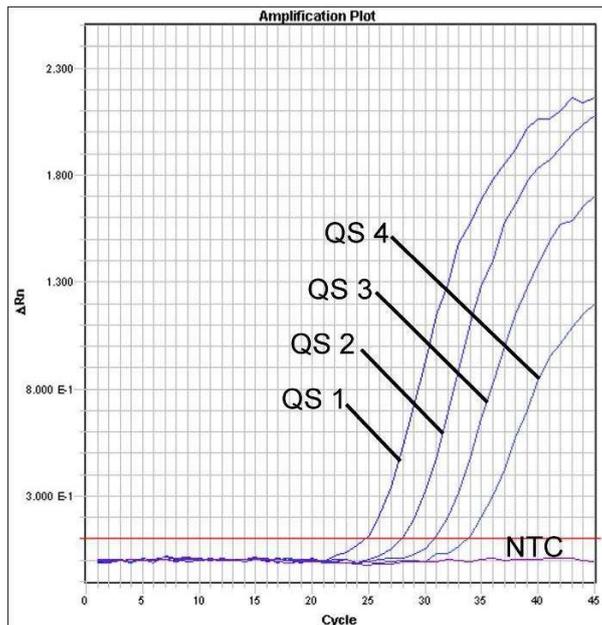


Fig. 26: Detection of the *Quantitation Standards (HSV2 LC/RG/TM QS 1 – 4)* by measurement of the NED fluorescence signal (**ABI PRISM 7900HT SDS**). NTC: non-template control (negative control).

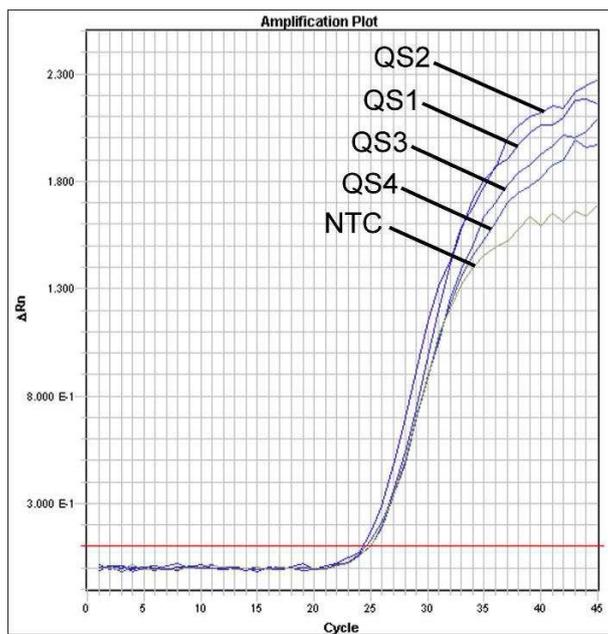


Fig. 27: Detection of the *Internal Control (IC)* by measurement of the VIC fluorescence signal (**ABI PRISM 7900HT SDS**) with simultaneous amplification of the *Quantitation Standards (HSV2 LC/RG/TM QS 1 – 4)*. NTC: non-template control (negative control).

## 10. Troubleshooting

### **Neither FAM fluorescence signal for HSV-1 nor NED fluorescence signal for HSV-2 with positive controls (HSV1 LC/RG/TM QS 1 – 4 & HSV2 LC/RG/TM 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 HSV-1 and NED for HSV-2 for the analytical HSV PCR and the detection dye VIC 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 7900HT SDS* see 8.5.2.4 *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 8.5 *Programming the ABI PRISM SDS*).
- Incorrect configuration of the PCR reaction.
  - ➔ Check your work steps by means of the pipetting scheme (see 8.4 *Preparing the PCR*) and repeat the PCR, if necessary.
- The storage conditions for one or more kit components did not comply with the instructions given in 2. Storage or the *artus HSV-1/2 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* (VIC fluorescence signal) and simultaneous absence of a FAM fluorescence signal for HSV-1 and NED fluorescence signal for HSV-2 for the specific HSV 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 a recommended isolation method (see 8.1 DNA Isolation) and stick closely to the manufacturer's instructions.
  - ➔ Make sure that during the DNA isolation the recommended additional centrifugation step has been carried out before the elution in order to remove any residual ethanol (see 8.1 DNA Isolation).
- DNA was lost during extraction.
  - ➔ If the *Internal Control* had been added to the extraction, an absent signal of the *Internal Control* can indicate the loss of DNA during the extraction. Make sure that you use a recommended isolation method (see 8.1 DNA Isolation) and stick closely to the manufacturer's instructions.
- The storage conditions for one or more kit components did not comply with the instructions given in 2. Storage or the *artus HSV-1/2 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 for HSV-1 and NED fluorescence signal for HSV-2 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 at last.
  - ➔ Make sure that work space and instruments are decontaminated at regular intervals.
- A contamination occurred during extraction.
  - ➔ Repeat the extraction and PCR of the sample to be tested using new reagents.
  - ➔ Make sure that work space and instruments are decontaminated at regular intervals.

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

# 11. Specifications

## 11.1 Analytical Sensitivity

In order to determine the analytical sensitivity of the *artus* HSV-1/2 TM PCR Kit, a standard dilution series has been set up for HSV-1 from 25.7 to nominal 0.008 HSV copy equivalents\*/ $\mu\text{l}$  and for HSV-2 from 35.31 to nominal 0.012 HSV copy equivalents/ $\mu\text{l}$  and analysed by means of the *ABI PRISM 7000* and *7900HT Sequence Detection Systems* with the help of the *artus* HSV-1/2 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. A graphical illustration of the probit analysis (*ABI PRISM 7900HT SDS*) is shown in Fig. 28 and 29.

| <b>Detection limit (<math>p = 0.05</math>)</b> |                           |
|--|---------------------------|
| <i>ABI PRISM 7000 SDS</i> (HSV-1)              | 0.9 copies/ $\mu\text{l}$ |
| <i>ABI PRISM 7000 SDS</i> (HSV-2)              | 0.5 copies/ $\mu\text{l}$ |
| <i>ABI PRISM 7900HT SDS</i> (HSV-1)            | 1.8 copies/ $\mu\text{l}$ |
| <i>ABI PRISM 7900HT SDS</i> (HSV-2)            | 2.0 copies/ $\mu\text{l}$ |

This means that there is a 95 % probability that 0.9 copies/ $\mu\text{l}$  (HSV-1) or 0.5 copies/ $\mu\text{l}$  (HSV-2, *ABI PRISM 7000 SDS*) and 1.8 copies/ $\mu\text{l}$  (HSV-1) or 2.0 copies/ $\mu\text{l}$  (HSV-2, *ABI PRISM 7900HT SDS*) will be detected.

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

### Probit analysis: HSV-1 (ABI PRISM 7900HT SDS)

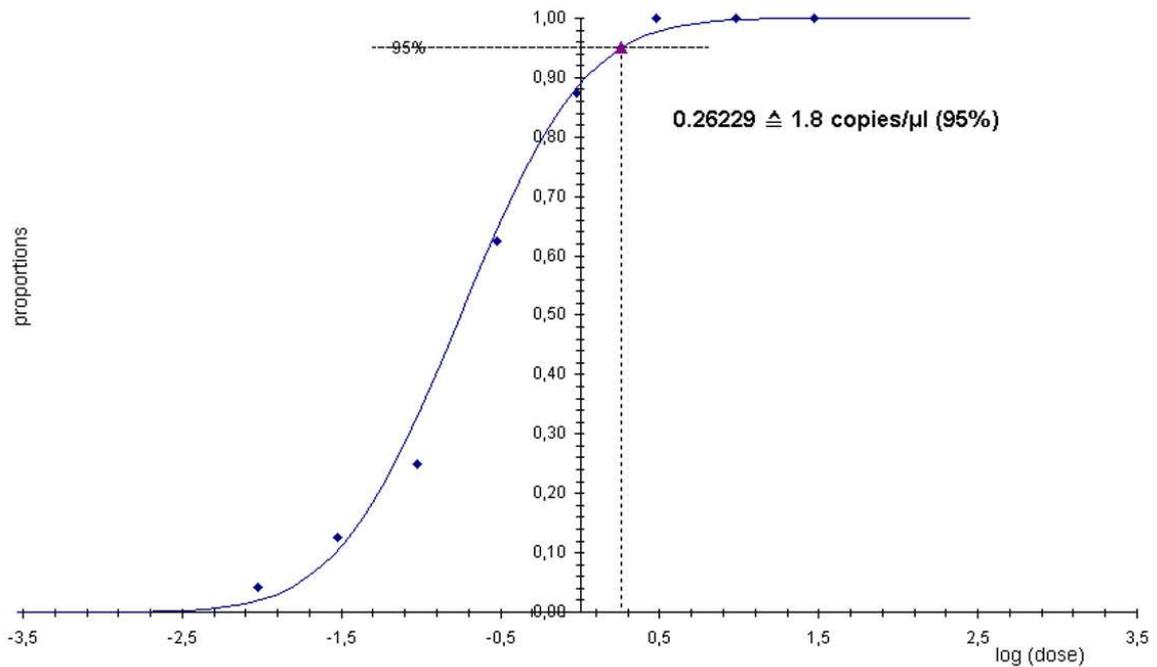


Fig. 28: Analytical sensitivity for **HSV-1** of the *artus* HSV-1/2 TM PCR Kit (*ABI PRISM 7900HT SDS*).

### Probit analysis: HSV-2 (ABI PRISM 7900HT SDS)

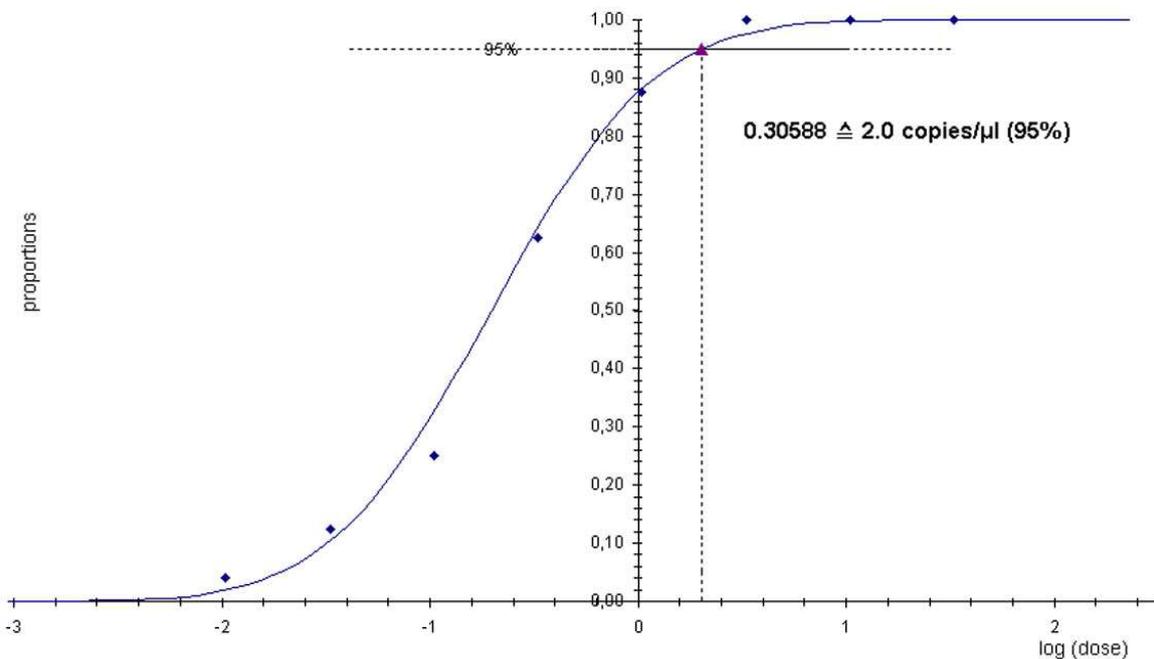


Fig. 29: Analytical sensitivity for **HSV-2** of the *artus* HSV-1/2 TM PCR Kit (*ABI PRISM 7900HT SDS*).

## 11.2 Specificity

The specificity of the *artus* HSV-1/2 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 in gene banks published sequences by sequence comparison analysis. The detectability of all relevant strains has thus been ensured.

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

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

| <b>Control group</b>                         | <b>HSV-1/2<br/>(FAM/NED)</b> | <b>Internal Control<br/>(VIC)</b> |
|--|------------------------------|-----------------------------------|
| Human herpesvirus 3 (Varicella-zoster virus) | –                            | +                                 |
| Human herpesvirus 4 (Epstein-Barr virus)     | –                            | +                                 |
| Human herpesvirus 5 (Cytomegalovirus)        | –                            | +                                 |
| Human herpesvirus 6 A                        | –                            | +                                 |
| Human herpesvirus 6 B                        | –                            | +                                 |
| Human herpesvirus 7                          | –                            | +                                 |
| Human herpesvirus 8                          | –                            | +                                 |
| Hepatitis A virus                            | –                            | +                                 |
| Hepatitis B virus                            | –                            | +                                 |
| Hepatitis C virus                            | –                            | +                                 |
| Human immunodeficiency virus                 | –                            | +                                 |
| Human T cell leukemia virus 1 and 2          | –                            | +                                 |
| West-Nile-virus                              | –                            | +                                 |
| Enterovirus                                  | –                            | +                                 |
| Parvovirus B19                               | –                            | +                                 |

### 11.3 Precision

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

Precision data of the *artus* HSV-1/2 TM PCR Kit have been collected using the *Quantitation Standard* of the lowest concentration (QS 4; 10 copies/ $\mu$ l). Testing was performed with eight replicates. The precision data were calculated on basis of the Ct values of the amplification curves (Ct: threshold cycle, see Table 2/Table 4). In addition, precision data for quantitative results in copies/ $\mu$ l were determined using the corresponding Ct values (see Table 3/Table 5). Based on these results, the overall statistical spread of any given sample with the mentioned concentration is 2.62 % (Ct; HSV-1) and 2.07 % (Ct; HSV-2) or 14.02 % (conc., HSV-1) and 14.82 % (conc., HSV-2), for the detection of the *Internal Control* 1.84 % (Ct; HSV-1) and 1.92 % (Ct; HSV-2). These values are based on the totality of all single values of the determined variabilities.

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

|   | <b>Standard deviation</b> | <b>Variance</b> | <b>Coefficient of variation [%]</b> |
|---|---------------------------|-----------------|-------------------------------------|
| Intra-assay variability:<br><i>HSV1 LC/RG/TM QS 4</i> | 0.18                      | 0.03            | 0.51                                |
| Intra-assay variability:<br><i>Internal Control</i>   | 0.24                      | 0.06            | 0.87                                |
| Inter-assay variability:<br><i>HSV1 LC/RG/TM QS 4</i> | 0.25                      | 0.06            | 0.70                                |
| Inter-assay variability:<br><i>Internal Control</i>   | 0.37                      | 0.14            | 1.36                                |
| Inter-batch variability:<br><i>HSV1 LC/RG/TM QS 4</i> | 0.23                      | 0.05            | 0.67                                |
| Inter-batch variability:<br><i>Internal Control</i>   | 0.17                      | 0.03            | 0.65                                |
| Total variance:<br><i>HSV1 LC/RG/TM QS 4</i>          | 0.92                      | 0.84            | 2.62                                |
| Total variance:<br><i>Internal Control</i>            | 0.49                      | 0.24            | 1.84                                |

**Table 3. Precision data for HSV-1 on basis of the quantitative results (in copies/ $\mu$ l)**

|   | <b>Standard deviation</b> | <b>Variance</b> | <b>Coefficient of variation [%]</b> |
|---|---------------------------|-----------------|-------------------------------------|
| Intra-assay variability:<br><i>HSV1 LC/RG/TM QS 4</i> | 1.32                      | 1.75            | 13.12                               |
| Inter-assay variability:<br><i>HSV1 LC/RG/TM QS 4</i> | 1.60                      | 2.56            | 15.81                               |
| Inter-batch variability:<br><i>HSV1 LC/RG/TM QS 4</i> | 1.22                      | 1.50            | 12.14                               |
| Total variance:<br><i>HSV1 LC/RG/TM QS 4</i>          | 1.42                      | 2.00            | 14.02                               |

## 11.4 Robustness

The verification of the robustness allows the determination of the total failure rate of the *artus* HSV-1/2 TM PCR Kit. 30 HSV negative samples of cerebrospinal fluid were spiked with 5.4 copies/ $\mu$ l elution volume of HSV-1 control DNA (threefold concentration of the analytical sensitivity limit). After extraction using the QIAamp DNA Mini Kit (see 8.1 DNA Isolation) these samples were analysed with the *artus* HSV-1/2 TM PCR Kit. The analysis of HSV-2 has been carried out similarly (30 cerebrospinal fluid samples, 6 copies/ $\mu$ l HSV-2 control DNA). For all HSV-1 and HSV-2 samples the failure rate was 0 %. In addition, the robustness of the *Internal Control* was assessed by purification and analysis of 30 HSV negative cerebrospinal fluid samples. The total failure rate was 0 %. Inhibitions were not observed. Thus, the robustness of the *artus* HSV-1/2 TM PCR Kit is  $\geq 99$  %.

## 11.5 Reproducibility

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

## 11.6 Diagnostic Evaluation

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

## 12. Product Use Limitations

- All reagents may exclusively be used in in vitro diagnostics.
- The product is to be used by personnel specially instructed and trained in the in vitro diagnostics procedures only.
- Strict compliance with the user manual is required for optimal PCR results.
- Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.

## 13. Warnings and Precautions

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

## 14. Quality Control

In accordance with QIAGEN's ISO 9001 and ISO 13485-certified Total Quality Management System, each lot of *artus* HSV-1/2 TM 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                       |
|   | Catalogue number                   |
|  | Material number                    |
|  | Handbook                           |
|  | In vitro diagnostic medical device |
|  | Global Trade Item Number           |
|  | Contains sufficient for <N> tests  |
|  | Temperature limitation             |
| <b>QS</b>   | <i>Quantitation Standard</i>       |
| <b>IC</b>   | <i>Internal Control</i>            |
| <b>Mg-Sol</b>   | <i>Magnesium Solution</i>          |

*artus* HSV-1/2 TM PCR Kit

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The *artus* HSV-1/2 TM PCR Kit, the BioRobot EZ1 Workstation, and the EZ1 DSP Virus Kit and Card are CE-marked diagnostic devices according to the European In Vitro Diagnostic Directive 98/79/EC. Not available in all countries.

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

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

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