

artus[®] HAV TM RT-PCR Kit v2.0

Handbook



24 (catalog no. 4516103)

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

For use with the

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

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artus HAV TM RT-PCR Kit v2.0

For use with the *ABI PRISM 7000, 7700 and 7900HT Sequence Detection Systems*.

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

Attention: The *artus HAV TM RT-PCR Kit v2.0* 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. 4516103 24 reactions |
|--------|--|----------------------------------|
| Blue | <i>HAV TM Master 1</i> | 2 x 12 rxns |
| Violet | <i>HAV TM Master 2</i> | 2 x 12 rxns |
| Red | <i>HAV TM QS 1st</i> <i>5 x 10³ IU/μl</i> | 1 x 200 μl |
| Red | <i>HAV TM QS 2nd</i> <i>5 x 10² IU/μl</i> | 1 x 200 μl |
| Red | <i>HAV TM QS 3rd</i> <i>5 x 10¹ IU/μl</i> | 1 x 200 μl |
| Red | <i>HAV TM QS 4th</i> <i>5 x 10⁰ IU/μl</i> | 1 x 200 μl |
| Green | <i>HAV TM ICst</i> | 1 x 1,000 μl |
| White | <i>Water (PCR grade)</i> | 1 x 1,000 μl |

▪ QS = Quantitation Standard
IC = Internal Control

2. Storage

The components of the *artus* HAV TM RT-PCR Kit v2.0 should be stored at -20°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
- RNA isolation kit (see **7.1 RNA 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 **7.4 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 **7.4 Preparing the PCR**
- Compression pad for use with optical adhesive covers (*Optical Cover Compression Pads*, Cat. No. 4 312 639, Applied Biosystems), see **7.4 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, 7700 or 7900HT SDS*

*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 **7.5.2 Programming the ABI PRISM 7700 SDS**, 7.5.2.5 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* HAV TM RT-PCR Kit v2.0 constitutes a ready-to-use system for the detection of HAV RNA using polymerase chain reaction (PCR) in the *ABI PRISM 7000, 7700 and 7900HT Sequence Detection System*. The *HAV TM Master 1 & 2* contain reagents and enzymes for the reverse transcription and specific amplification of a 90 bp region of the HAV genome. The amplicon is detected by measuring the FAM fluorescence in the *ABI PRISM SDS*. In addition, the *artus* HAV TM RT-PCR Kit v2.0 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 HAV RT PCR (see **10.1 Analytical Sensitivity**) is not reduced. External positive controls (*HAV TM QS 1 - 4*) are supplied which allow the determination of the pathogen load. For further information, please refer to section **7.3 Quantitation**.

7. Protocol

7.1 RNA Isolation

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

| Sample Material | Nucleic Acid Isolation Kit | Catalogue Number | Manufacturer | Carrier RNA |
|-----------------|-----------------------------------|------------------|--------------|--------------|
| Serum, plasma | QIAamp® UltraSens® Virus Kit (50) | 53 704 | QIAGEN | included |
| | QIAamp Viral RNA Mini Kit (50) | 52 904 | QIAGEN | included |
| | QIAamp DSP Virus Kit (50) | 60 704 | QIAGEN | included |
| | QIAamp DNA Stool Mini Kit (50) | 51 504 | QIAGEN | non included |

- 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 Viral RNA Mini Kit/QIAamp UltraSens Virus Kit/QIAamp DSP Virus Kit, we recommend the following procedure:
 - a. Resuspend the lyophilised carrier RNA prior to first use of the extraction kit in 310 µl of the elution buffer provided with the kit (final concentration 1 µg/µl, do not use lysis buffer). Portion this carrier RNA solution into a number of aliquots adequate to your needs and store them at -20°C. Avoid repeated thawing (> 2 x) of a carrier RNA aliquot.
 - b. Before the beginning of each extraction, a mixture of lysis buffer and carrier RNA (and *Internal Control*, where applicable, see **7.2 Internal Control**) should be prepared freshly according to the following pipetting scheme:

For use of **QIAamp Viral RNA Mini Kit**:

| Number of samples | 1 | 12 |
|------------------------------|-----------------|--------------------|
| Lysis buffer AVL | 560 µl | 6,720 µl |
| Carrier RNA (1 µg/µl) | 5.6 µl | 67.2 µl |
| Total Volume | 565.6 µl | 6,787.2 µl |
| Volume per extraction | 560 µl | each 560 µl |

For use of **QIAamp UltraSens Virus Kit**:

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

For use of **QIAamp DSP Virus Kit**:

| Number of samples | 1 | 12 |
|------------------------------|-----------------|--------------------|
| Lysis buffer AL | 500 µl | 6,000 µl |
| Carrier RNA (1 µg/µl) | 5.6 µl | 67.2 µl |
| Total Volume | 505.6 µl | 6,067.2 µl |
| Volume per extraction | 500 µl | each 500 µl |

- c. Please use the freshly prepared mixture of lysis buffer and carrier RNA instantly for extraction. Storage of the mixture is not possible.
- The **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* HAV TM RT-PCR Kit v2.0 should not be used with **phenol**-based isolation methods.

Important: The *Internal Control* of the *artus* HAV TM RT-PCR Kit v2.0 can be used directly in the isolation procedure (see **7.2 Internal Control**).

7.2 Internal Control

An *Internal Control* (*HAV TM IC*) is supplied. This allows the user **both to control the RNA 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. For example, using the QIAamp UltraSens Virus Kit the RNA is eluted in 60 µl AVE buffer. Hence, 6 µl of the *Internal Control* should be added initially. If you elute e.g. in 50 µl, then use the corresponding volume of 5 µl. The quantity of *Internal Control* used depends **only** on the elution volume. The *Internal Control* and carrier RNA (see **7.1 RNA 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 µl of the *Internal Control* per reaction directly to the mixture of *HAV TM Master 1 & 2* (30 µl). 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 mixture of

* 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.

HAV TM Master 1 & 2 and the Internal Control according to the number of samples (see 7.4 Preparing the PCR).

7.3 Quantitation

The enclosed *Quantitation Standards (HAV TM 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 7.5 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.

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

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

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).

In order to convert the values determined into IU/mg of sample material please apply the following equation:

$$\text{Result (IU/mg)} = \frac{\text{Result (IU/}\mu\text{l)} \times \text{Elution Volume (}\mu\text{l)}}{\text{Sample Material (mg)}}$$

For this purpose it is important to determine the initial weight of the sample before starting the isolation procedure.

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*).

7.4 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 | <i>ABI PRISM</i> Optical Tubes, 8 Tubes/Strip | 4 316 567 | Applied Biosystems | yes | – |
| Optical reaction tubes | MicroAmp [®] Optical Tubes | N8010933 | Applied Biosystems | yes | – |
| Optical caps (flat) | <i>ABI PRISM</i> 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 **7.5.2 Programming the *ABI PRISM 7700 SDS***, 7.5.2.5 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*, *PCR grade*) are performed in parallel. To generate a standard curve, use all supplied *Quantitation Standards (HAV TM QS 1 - 4)* for each PCR run. Before each use, all reagents need to be thawed completely, mixed (by repeated up

* 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.

and down pipetting or by inverting the tube several times) and centrifuged briefly.

If you want to use the *Internal Control* to monitor the RNA isolation procedure and to check for possible PCR inhibition, it has already been added to the isolation (see 7.2 Internal Control). In this case, please use the following pipetting scheme (for a schematic overview see Fig. 1):

| | | Number of samples | 1 | 12 |
|------------------------------|------------------------|-------------------|--------------|-------------------|
| 1. Preparation of Master Mix | <i>HAV TM Master 1</i> | | 25 µl | 300 µl |
| | <i>HAV TM Master 2</i> | | 5 µl | 60 µl |
| | <i>HAV TM IC</i> | | 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 mixture of *HAV TM Master 1* & 2. 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 | <i>HAV TM Master 1</i> | | 25 µl | 300 µl |
| | <i>HAV TM Master 2</i> | | 5 µl | 60 µl |
| | <i>HAV TM IC</i> | | 2 µl | 24 µl |
| | Total Volume | | 32 µl* | 384 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 |

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 RNA isolation. Mix the solution well by repeated up and down pipetting. Close

* 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.

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 **7.5 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, 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 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

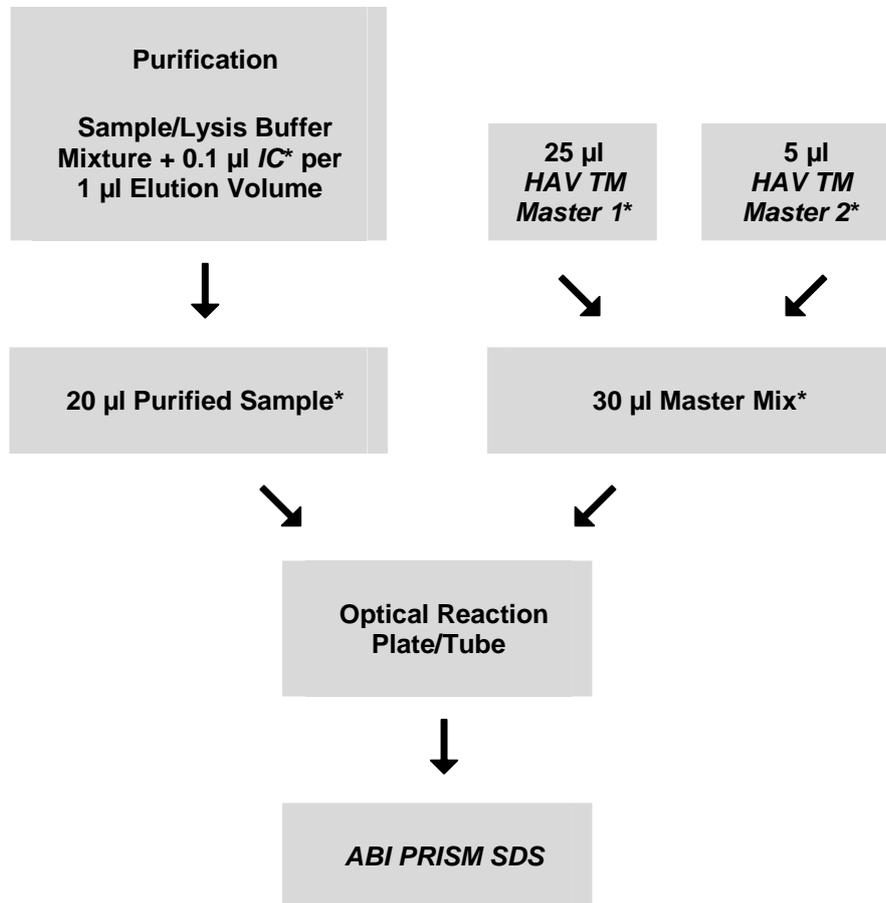


Fig. 1: Schematic workflow for the control of both the purification procedure and PCR inhibition.

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

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

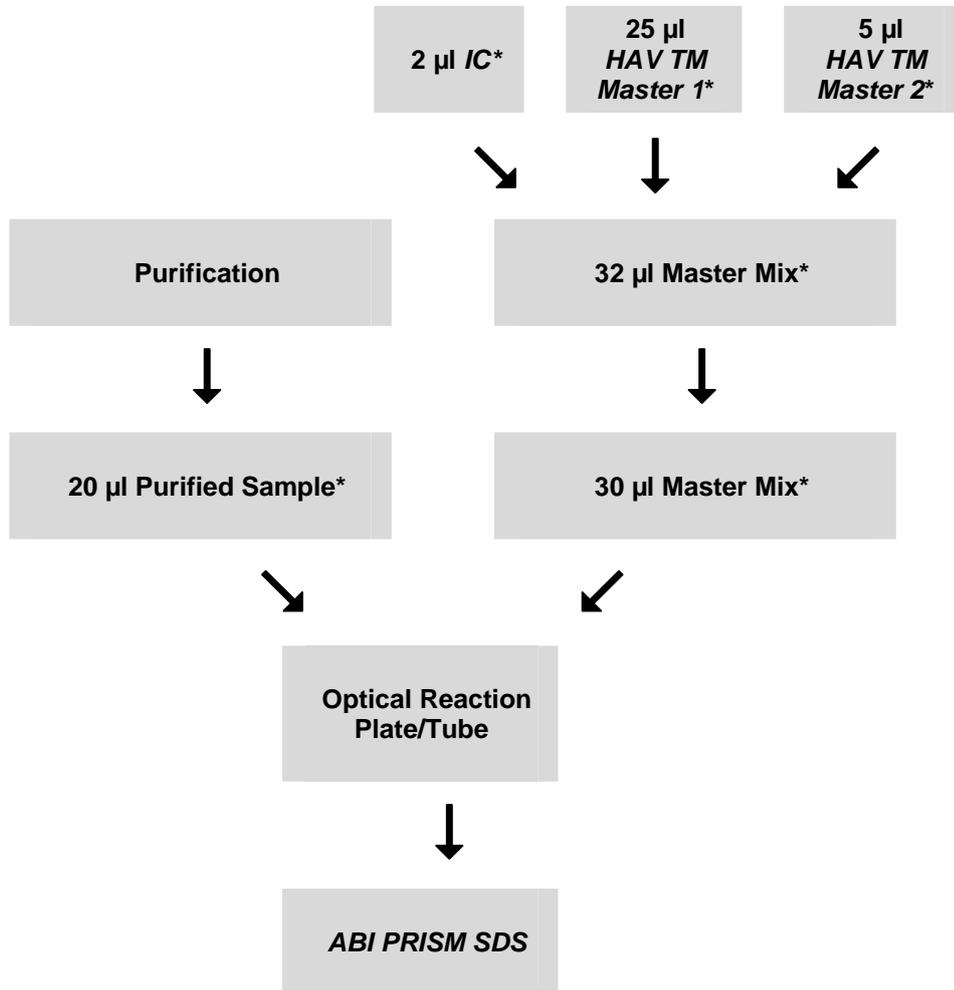


Fig. 2: Schematic workflow for the control of PCR inhibition.

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

7.5 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.

7.5.1 Programming the *ABI PRISM 7000 SDS*

For the detection of HAV RNA, create a profile on your *ABI PRISM 7000 SDS* according to the following six steps (7.5.1.1 - 7.5.1.6). 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.

7.5.1.1 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 [*.sdf]*) is available from the *Template* list or by selection using the *Browse* function (see **7.5.1.5 Saving the PCR Run**). Confirm your pre-settings (*OK*).

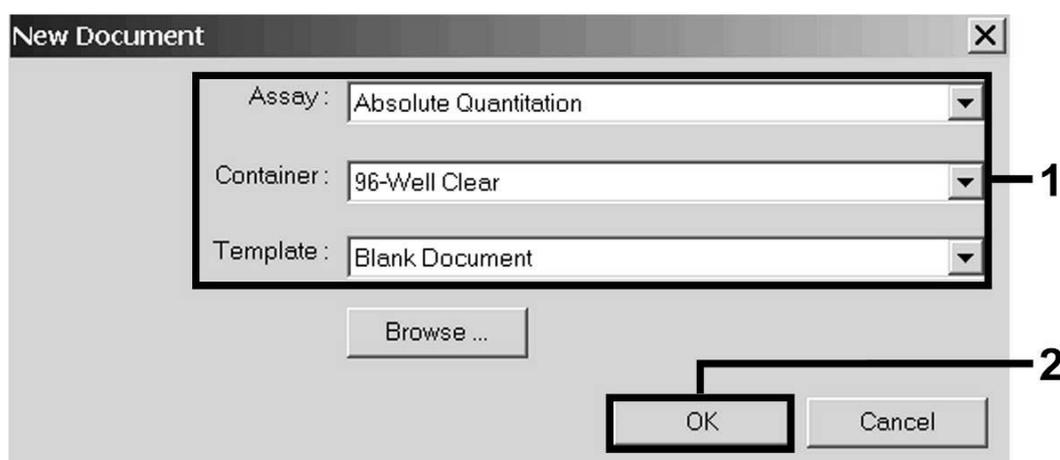


Fig. 3: Pre-settings for creating a new PCR run (*New Document*).

7.5.1.2 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 HAV RNA as well as the *Internal Control* by means of the *artus HAV TM RT-PCR Kit v2.0*, the reporters/quenchers listed in the following table are to be defined:

| Detection | Reporter | Quencher |
|-------------------------------------|----------|----------|
| HAV RNA | FAM | none |
| <i>Internal Control (HAV TM IC)</i> | VIC | none |

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

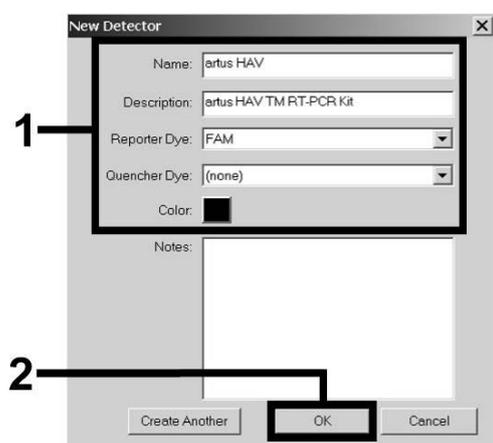


Fig. 4: Creating the HAV specific detector (*Detector Manager*).

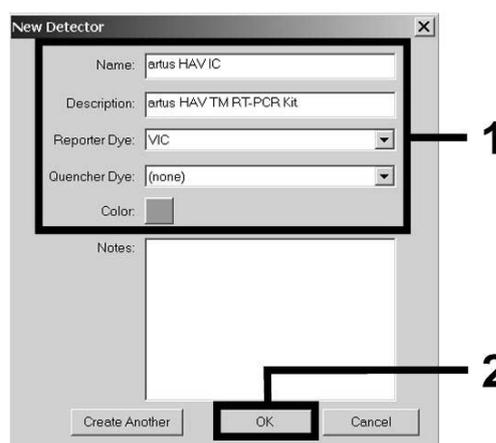


Fig. 5: Creating the *IC* specific detector (*Detector Manager*).

For the detection of HAV RNA 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*).

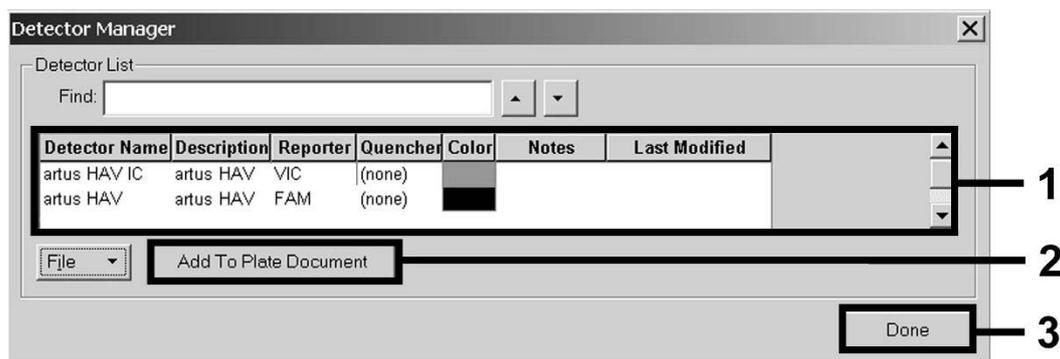


Fig. 6: Selecting the detectors (*Detector Manager*).

7.5.1.3 Assigning the Necessary Information to the Plate Positions

Open *Well Inspector* from the *View* menu to find the detectors selected under 7.5.1.2 (see Fig. 7).

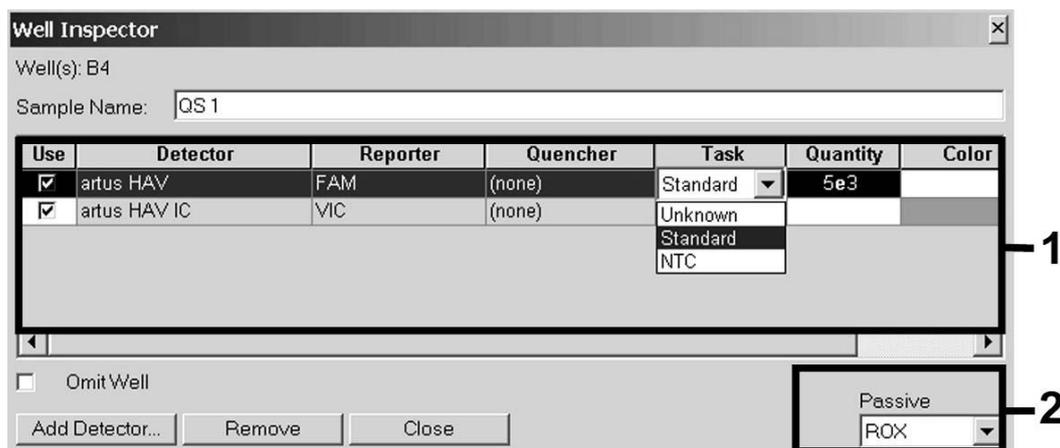


Fig. 7: Assigning the necessary information to the plate positions (*Well Inspector*).

Mark the plate positions reserved for the detection of HAV RNA. 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* (*HAV 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 HAV TM RT-PCR Kit v2.0*, **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 *HAV TM Master 1 & 2* guarantees the recognition and calculation of tube-to-tube variations (fluorescence differences between several PCR preparations) by means of the *Sequence Detection Software* (normalization).

7.5.1.4 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 HAV RNA according to Fig. 8. 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*).

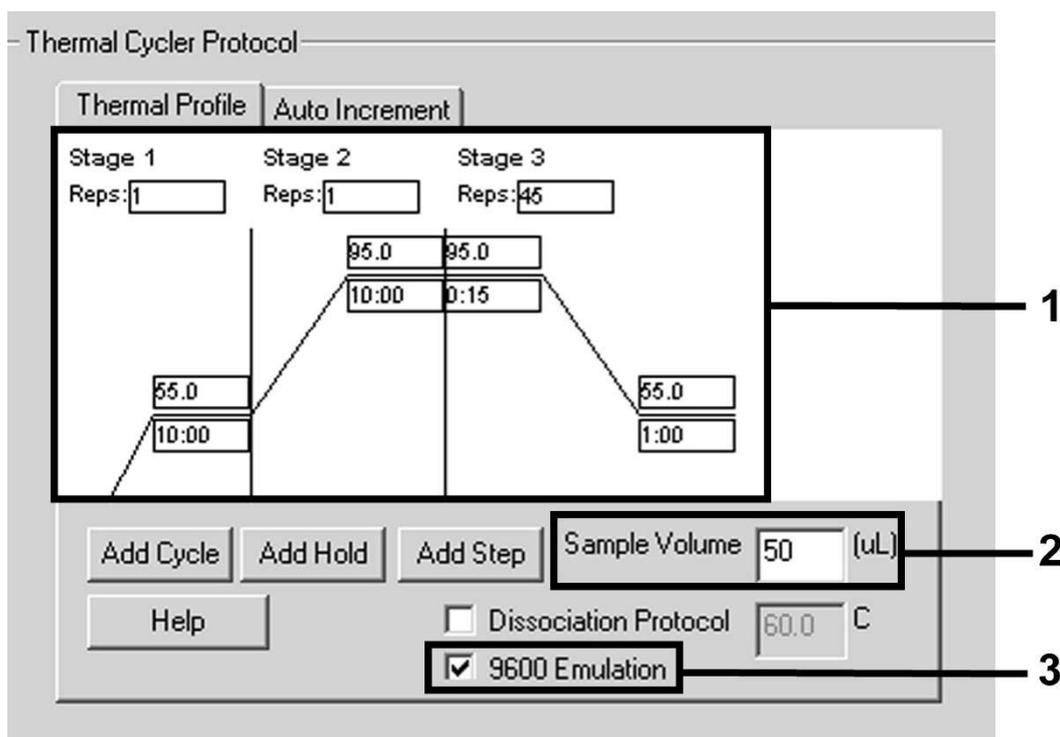


Fig. 8: Creating the temperature profile.

7.5.1.5 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.

7.5.1.6 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.

7.5.2 Programming the *ABI PRISM 7700 SDS*

For the detection of HAV RNA, create a profile on your *ABI PRISM 7700 SDS* according to the following seven steps (7.5.2.1 - 7.5.2.7). 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.

7.5.2.1 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*).

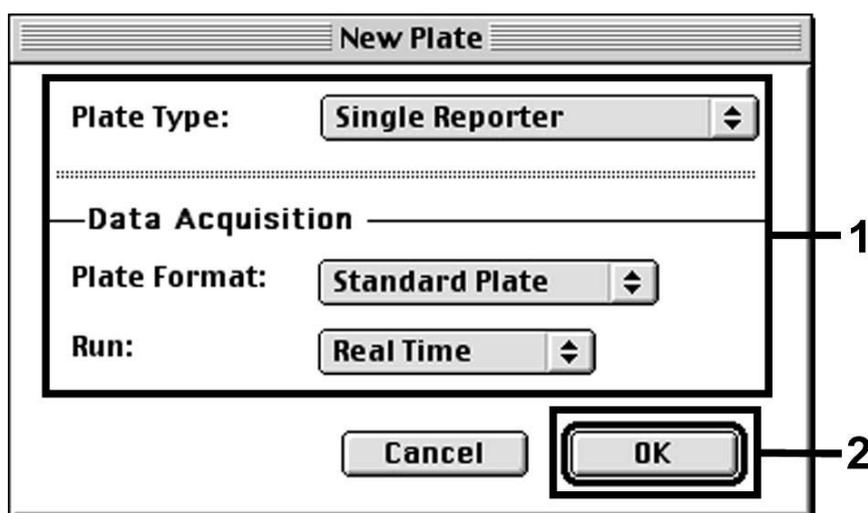


Fig. 9: Pre-settings for creating a new PCR run (*New Plate*).

7.5.2.2 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 HAV RNA as well as the *Internal Control* by means of the *artus HAV TM RT-PCR Kit v2.0*, the reporters/quenchers listed in the following table are to be defined:

| Detection | Reporter | Quencher |
|-------------------------------------|----------|----------|
| HAV RNA | FAM | TAMRA |
| <i>Internal Control (HAV TM IC)</i> | VIC | TAMRA |

For the analysis of HAV RNA by means of the *artus* HAV TM RT-PCR Kit v2.0, 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 **VIC** as reporter. As quencher set **TAMRA**. The assignment of the dyes and sample types in the window *Sample Type Setup* is shown in Fig. 10.

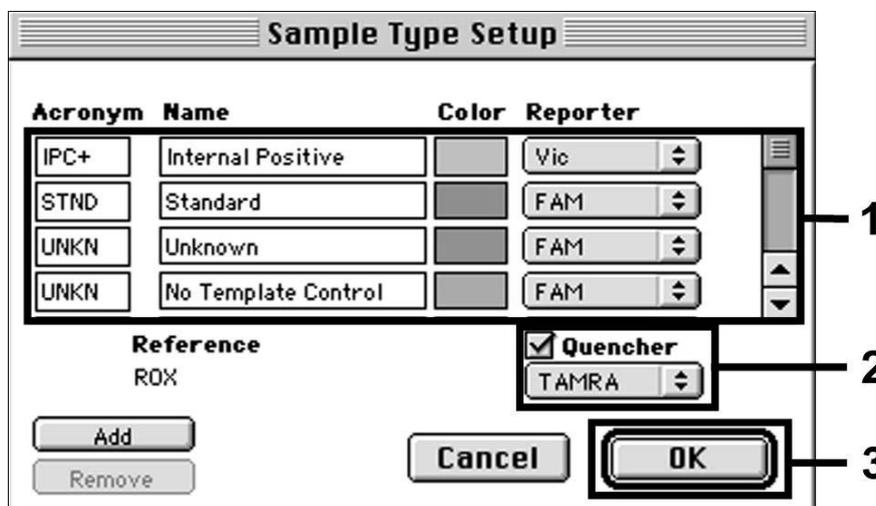


Fig. 10: Selecting the fluorescence dyes and assigning the sample type (*Sample Type Setup*).

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

| Sample Type | Function (<i>Acronym</i>) | Concentration (<i>Quantity</i>) | Reporter | Quencher |
|----------------------|-----------------------------|-----------------------------------|----------|----------|
| Sample | UNKN | - | FAM | TAMRA |
| Non-template control | UNKN | - | FAM | TAMRA |
| Standard | STND | see 1. Contents | FAM | TAMRA |

7.5.2.3 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 7.5.2.2) 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.

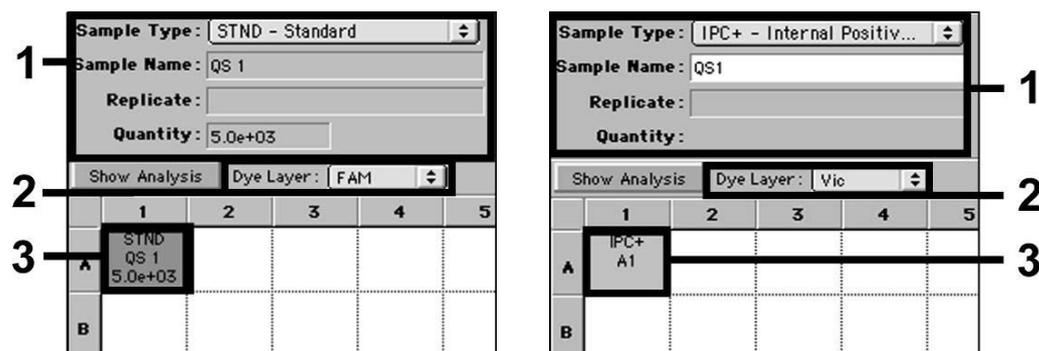


Fig. 11/12: Assigning the necessary information to the plate positions.

To generate a standard curve, use all supplied *Quantitation Standards* (*HAV TM 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.

7.5.2.4 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 HAV RNA 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).

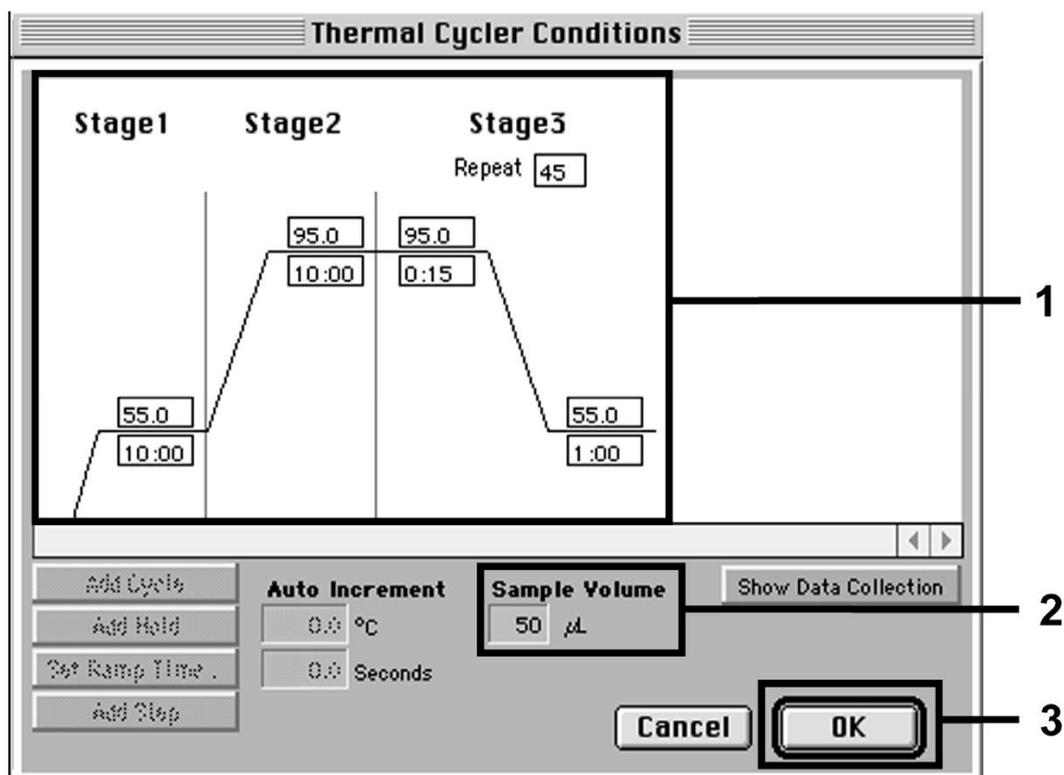


Fig. 13: Creating the temperature profile.

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 (*Stage3/Step2*) in order to exclude unnecessary fluorescence measurements. The total run time and the data quantity will thus be kept at a minimum.

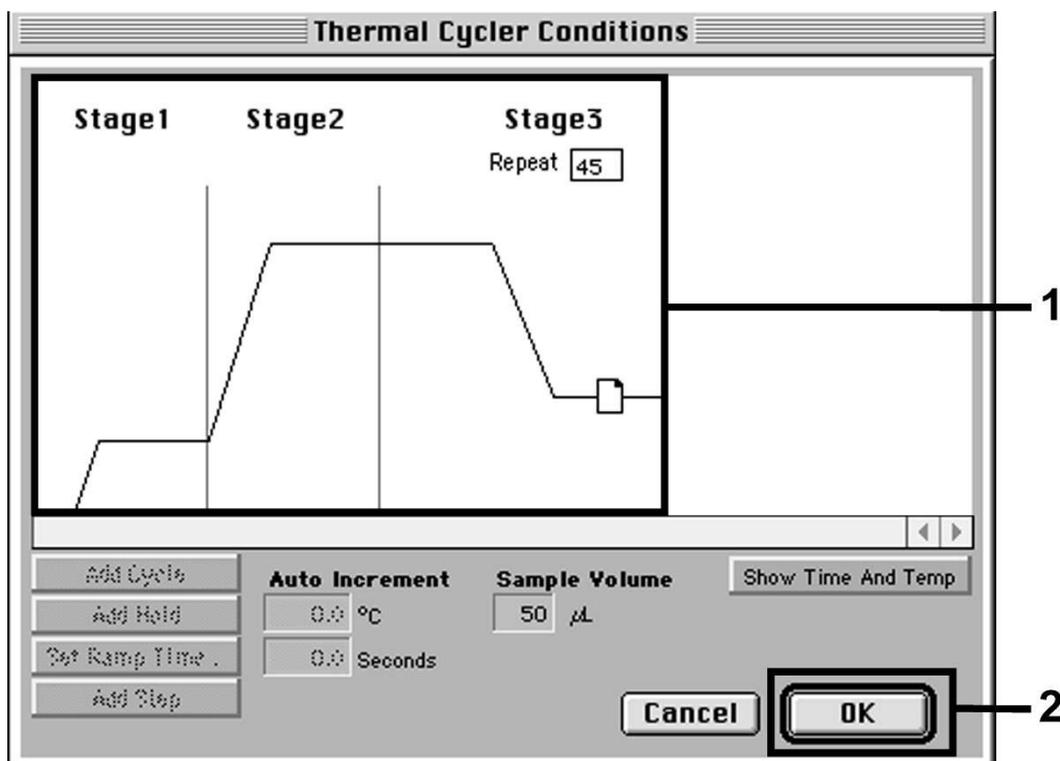


Fig. 14: Data collection.

7.5.2.5 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 HAV TM RT-PCR Kit v2.0*, **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 *HAV TM Master 1 & 2* 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**.

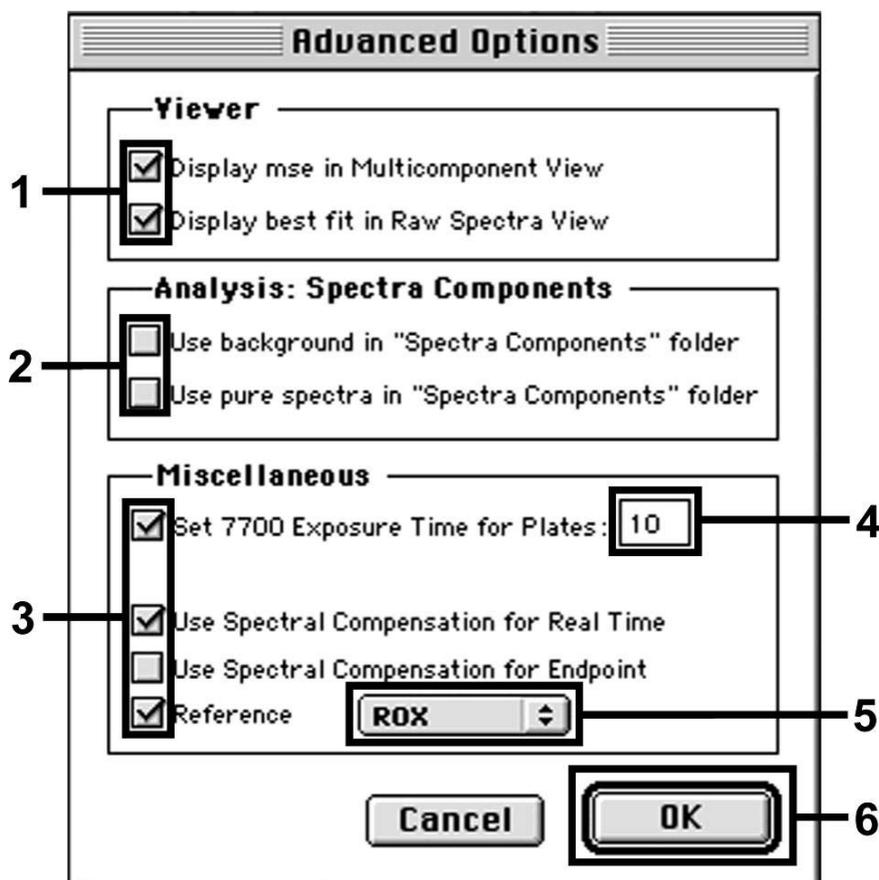


Fig. 15: Important additional settings (*Advanced Options*).

7.5.2.6 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.

7.5.2.7 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.

7.5.3 Programming the *ABI PRISM 7900HT SDS*

For the detection of HAV RNA, create a profile on your *ABI PRISM 7900HT SDS* according to the following six steps (7.5.3.1 - 7.5.3.6). 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.

7.5.3.1 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 [*].sdf*) is available from the *Template* list or by selection using the *Browse* function (see **7.5.3.5 Saving the PCR Run**). Confirm the pre-settings (*OK*).

Attention: The *artus* HAV TM RT-PCR Kit v2.0 may not be used in combination with the 384 plate format of the *ABI PRISM 7900HT SDS*.

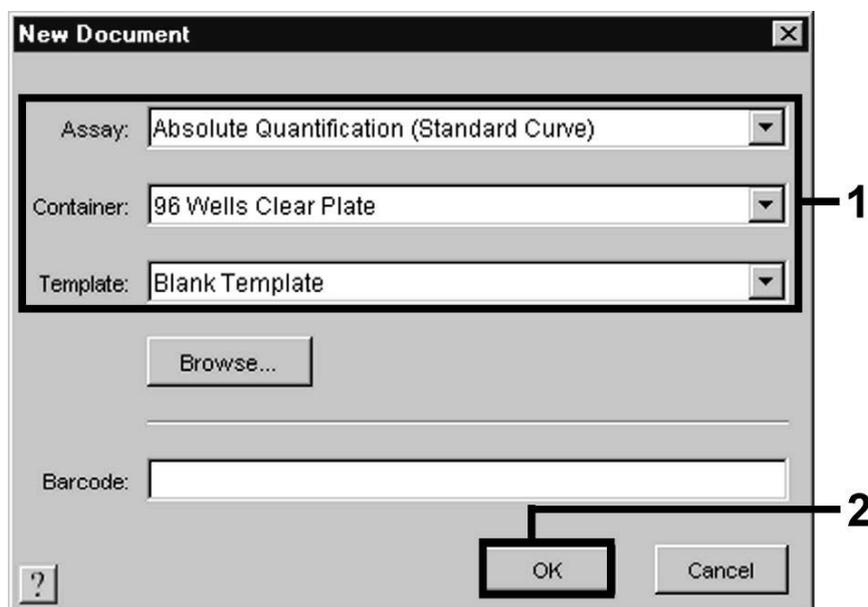


Fig. 16: Pre-settings for creating a new PCR run (*New Document*).

7.5.3.2 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 HAV RNA as well as the *Internal Control* by means of the *artus HAV TM RT-PCR Kit v2.0*, the reporters/quenchers listed in the following table are to be defined:

| Detection | Reporter | Quencher |
|-------------------------------------|----------|-----------------|
| HAV RNA | FAM | Non Fluorescent |
| <i>Internal Control (HAV TM IC)</i> | VIC | Non Fluorescent |

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

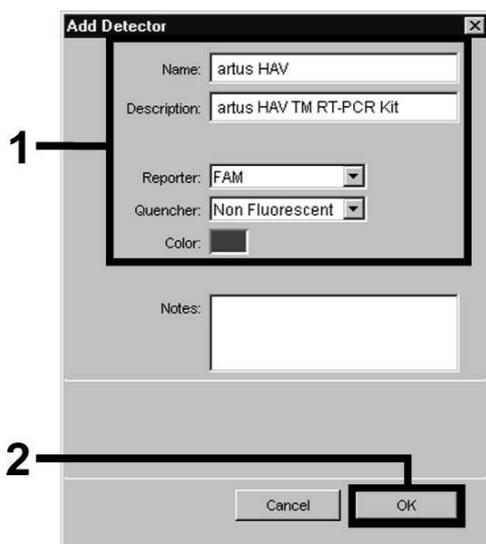


Fig. 17: Creating the HAV specific detector (*Detector Manager*).

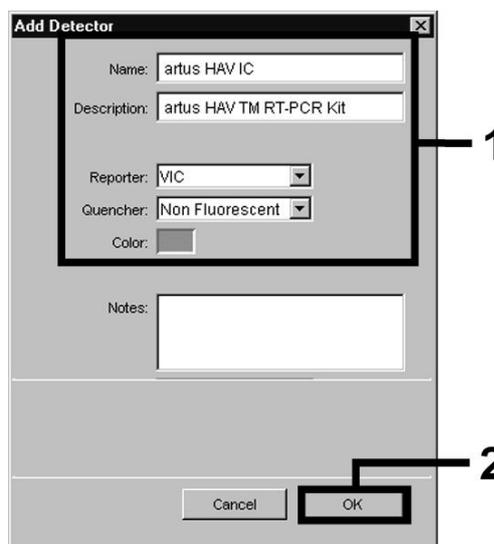


Fig. 18: Creating the IC specific detector (*Detector Manager*).

For the detection of HAV RNA 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*).

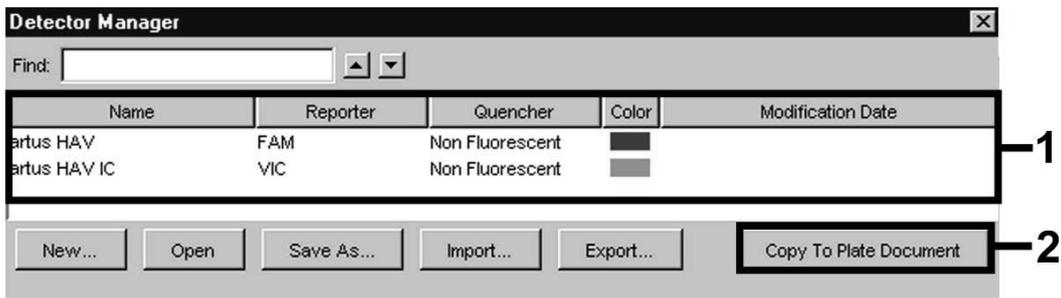


Fig. 19: Selecting the detectors (*Detector Manager*).

7.5.3.3 Assigning the Necessary Information to the Plate Positions

After having closed the *Detector Manager* (*Done*) the detectors selected under 7.5.3.2 are found on the *Setup* level listed in a table (see Fig. 20).

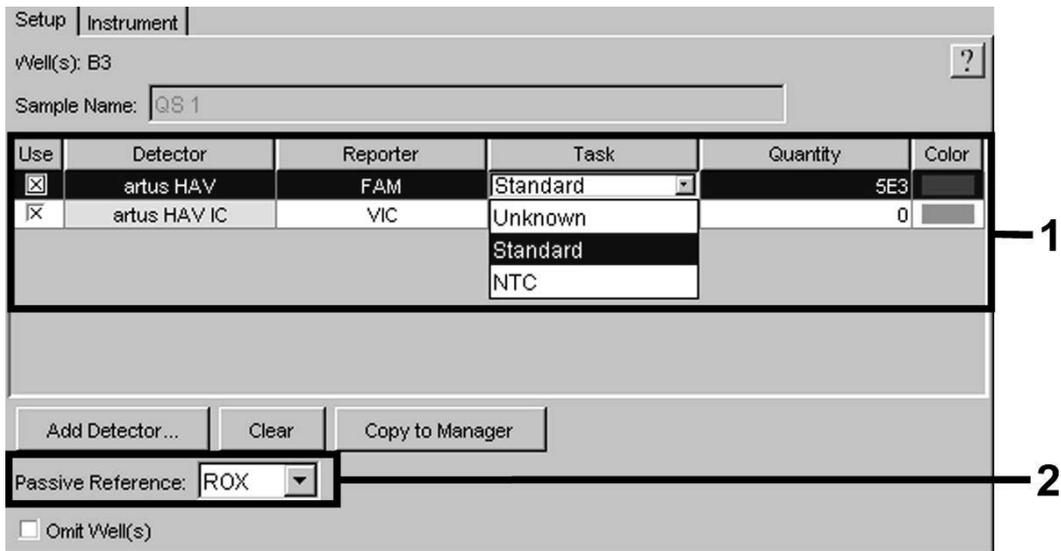


Fig. 20: Assigning the necessary information to the plate positions.

Mark the plate positions reserved for the detection of HAV RNA. 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* (*HAV 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 HAV TM RT-PCR Kit v2.0*, **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 *HAV TM Master 1 & 2* guarantees the recognition and calculation of tube-to-tube variations (fluorescence differences between several PCR preparations) by means of the *Sequence Detection Software* (normalization).

7.5.3.4 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 HAV RNA 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*).

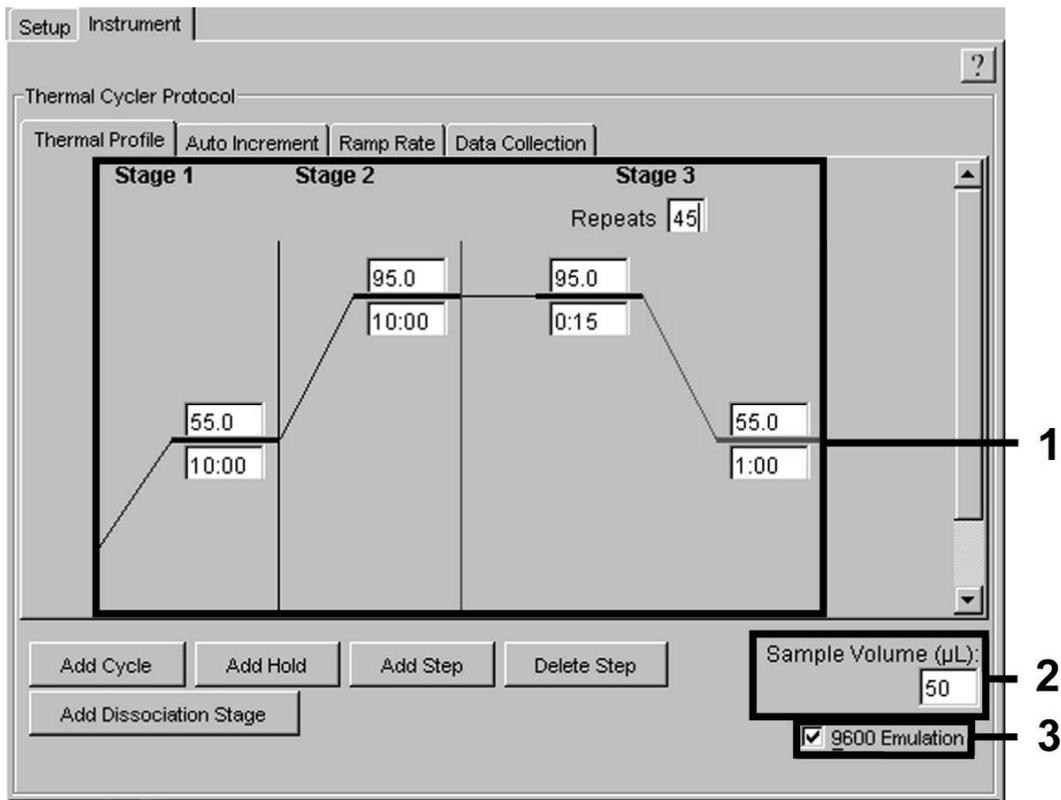


Fig. 21: Creating the temperature profile.

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 (*Stage3/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.

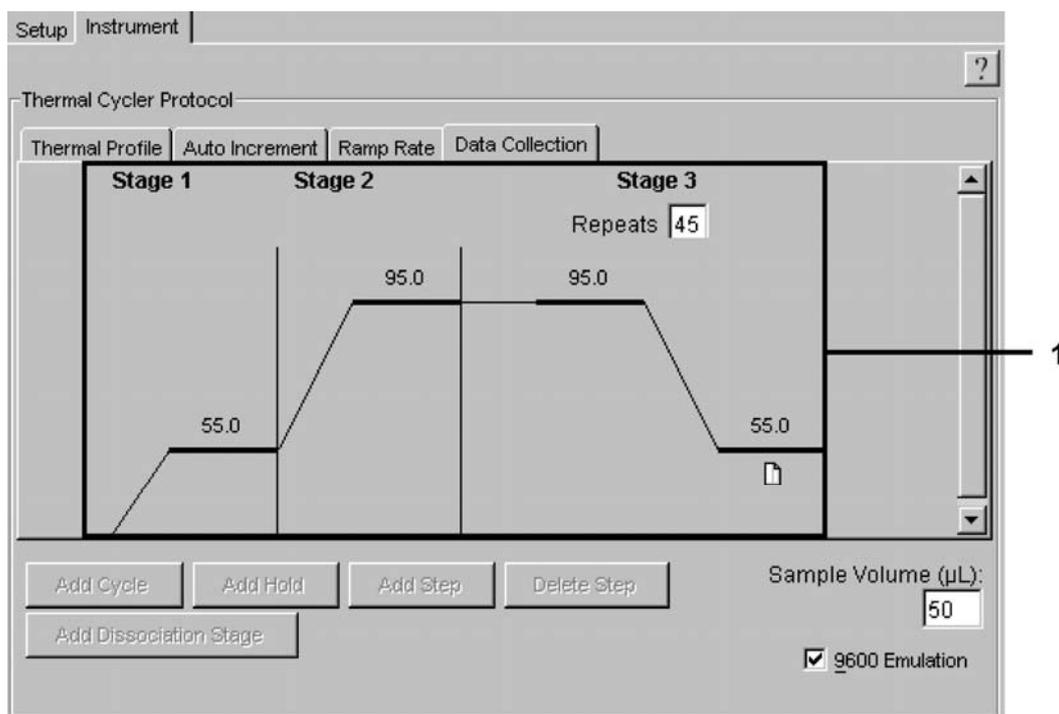


Fig. 22: Data collection.

7.5.3.5 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.

7.5.3.6 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*™ 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 is detected.

The result of the analysis is positive: The sample contains HAV RNA.

In this case, the detection of a VIC fluorescence signal (*Internal Control*) is dispensable, since high initial concentrations of HAV RNA (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 fluorescence signal from the *Internal Control* appears.

In the sample no HAV RNA is detectable. It can be considered negative.

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

3. Neither a FAM fluorescence signal nor a VIC 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**).

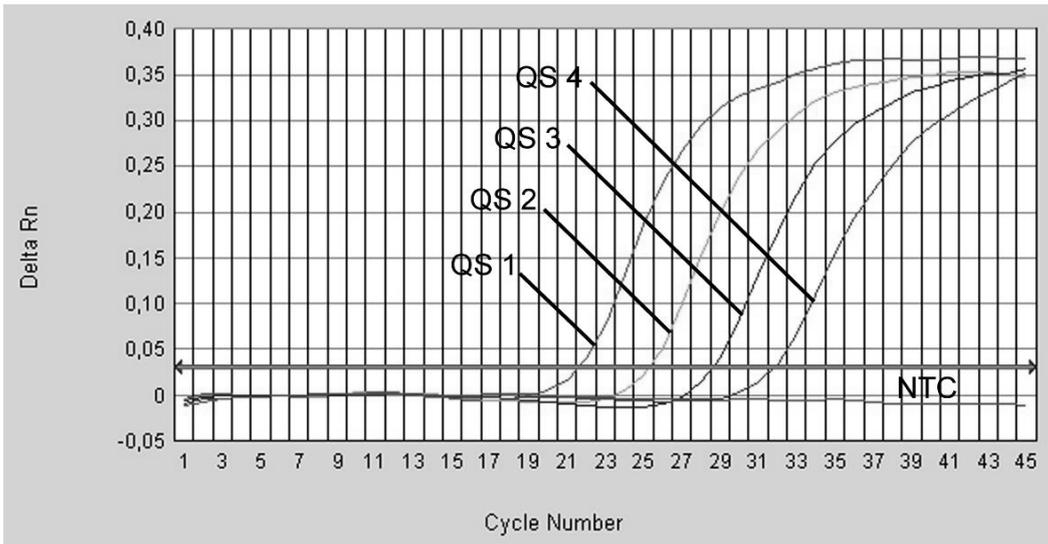


Fig. 23: Detection of the *Quantitation Standards (HAV TM QS 1 - 4)* by measurement of the FAM fluorescence signal (**ABI PRISM 7000 SDS**). NTC: non-template control (negative control).

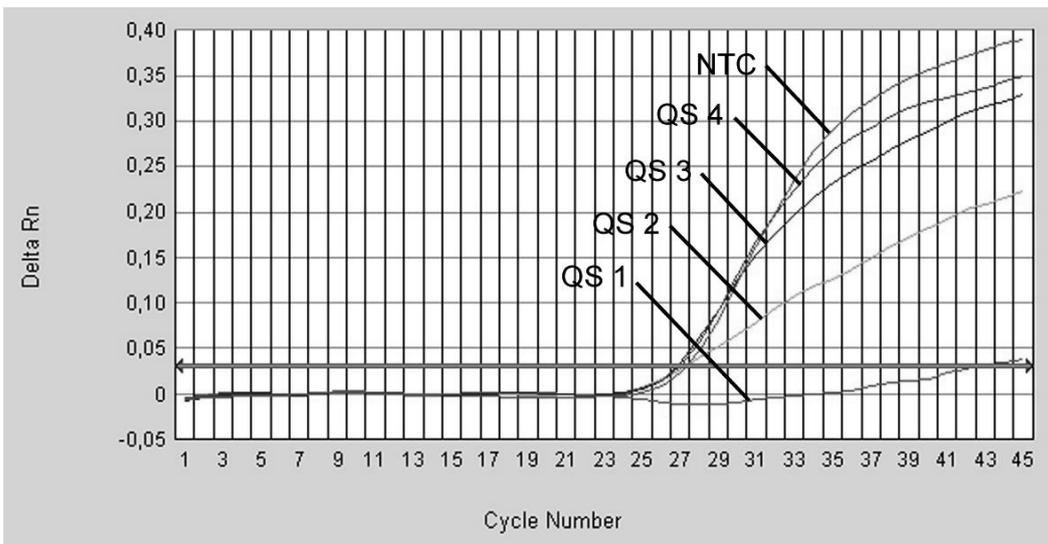


Fig. 24: Detection of the *Internal Control (IC)* by measurement of the VIC fluorescence signal (**ABI PRISM 7000 SDS**) with simultaneous amplification of the *Quantitation Standards (HAV TM QS 1 - 4)*. NTC: non-template control (negative control).

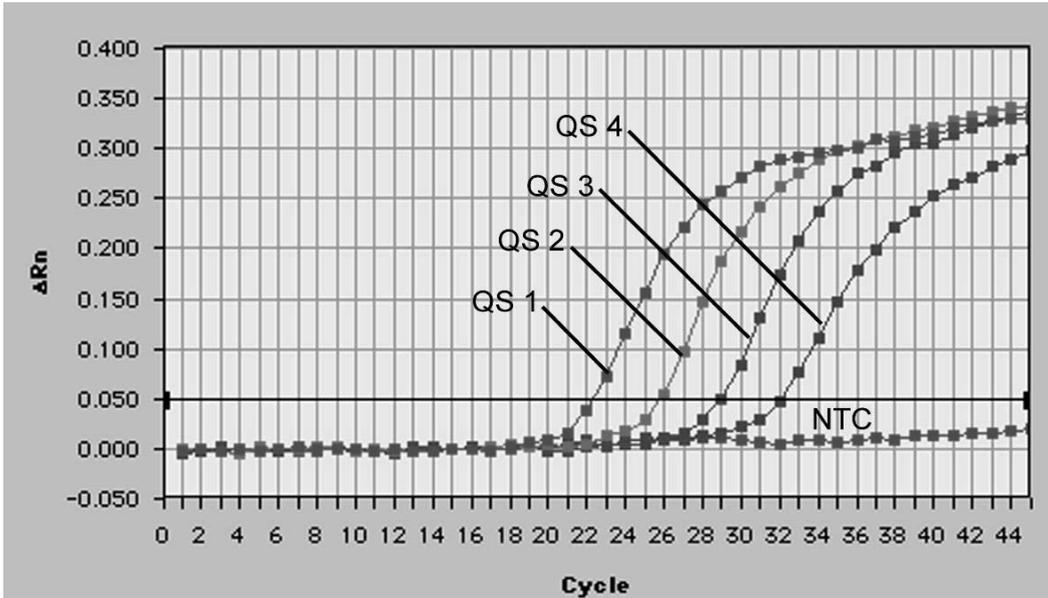


Fig. 25: Detection of the *Quantitation Standards (HAV TM QS 1 - 4)* by measurement of the FAM fluorescence signal (**ABI PRISM 7700 SDS**). NTC: non-template control (negative control).

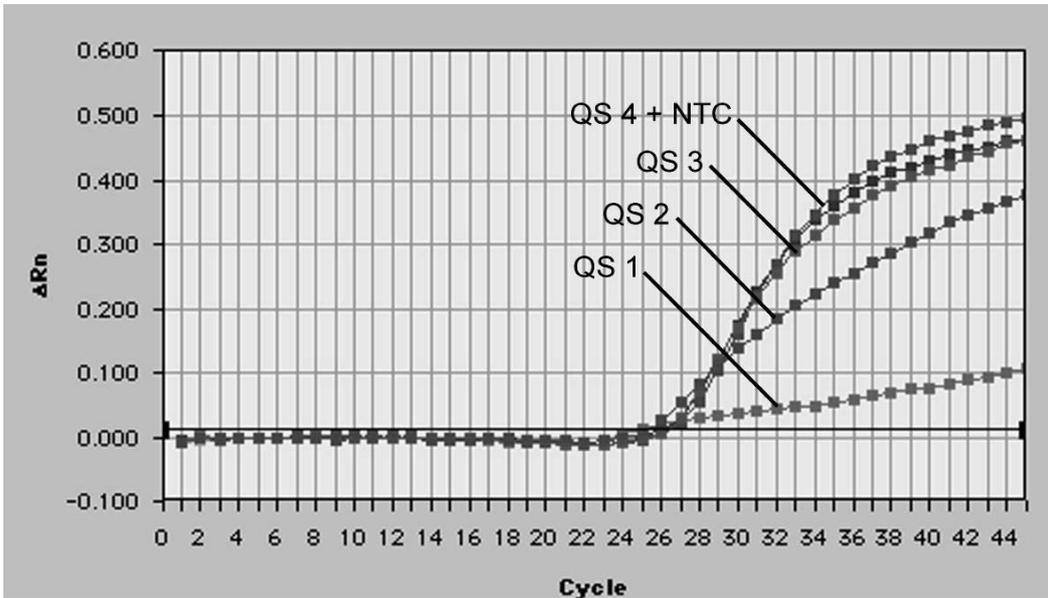


Fig. 26: Detection of the *Internal Control (IC)* by measurement of the VIC fluorescence signal (**ABI PRISM 7700 SDS**) with simultaneous amplification of the *Quantitation Standards (HAV TM QS 1 - 4)*. NTC: non-template control (negative control).

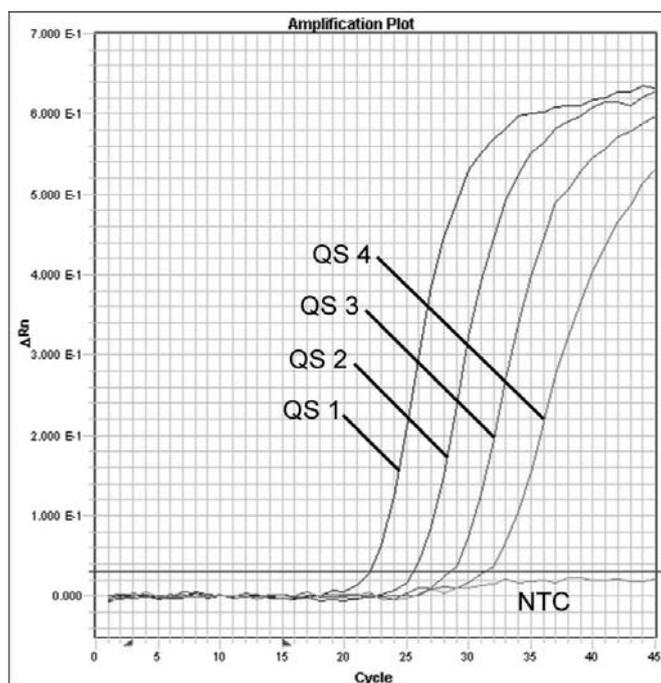


Fig. 27: Detection of the *Quantitation Standards (HAV TM QS 1 - 4)* by measurement of the FAM fluorescence signal (*ABI PRISM 7900HT SDS*). NTC: non-template control (negative control).

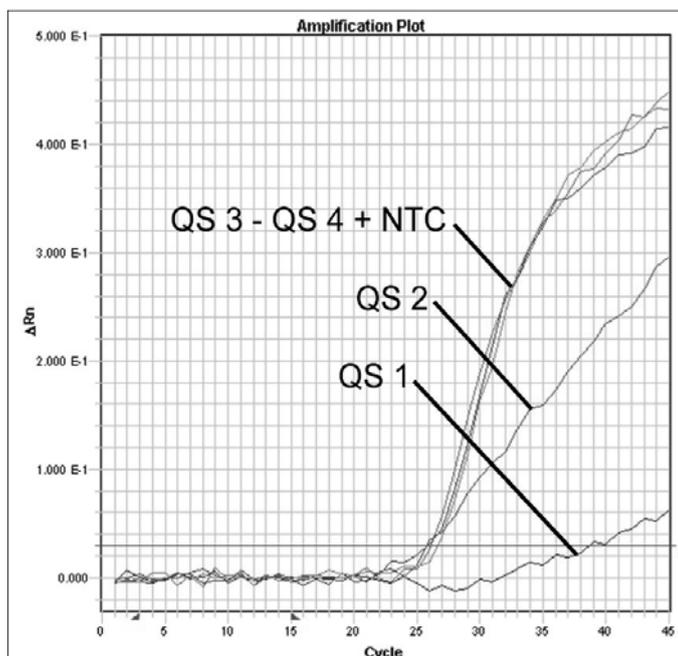


Fig. 28: Detection of the *Internal Control (IC)* by measurement of the VIC fluorescence signal (*ABI PRISM 7900HT SDS*) with simultaneous amplification of the *Quantitation Standards (HAV TM QS 1 - 4)*. NTC: non-template control (negative control).

9. Troubleshooting

No FAM fluorescence signal with positive controls (*HAV 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 the analytical HAV RT PCR and the detection dye VIC for the *Internal Control* RT 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 **7.5.2.4 Creating the Temperature Profile**, for *ABI PRISM 7900HT SDS* see **7.5.3.4 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 **7.5 Programming the ABI PRISM SDS**).
- Incorrect configuration of the PCR reaction.
 - Check your work steps by means of the pipetting scheme (see **7.4 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 HAV TM RT-PCR Kit v2.0* 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 the specific HAV RT 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 **7.1 RNA Isolation**) and stick closely to the manufacturer's instructions.
 - Make sure that during the RNA isolation the recommended additional centrifugation step has been carried out before the elution in order to remove any residual ethanol (see **7.1 RNA Isolation**).
- RNA 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 RNA during the extraction. Make sure that you use a recommended isolation method (see **7.1 RNA 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* HAV TM RT-PCR Kit v2.0 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 RT 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.

10. Specifications

10.1 Analytical Sensitivity

In order to determine the analytical sensitivity of the *artus* HAV TM RT-PCR Kit v2.0, a dilution series of the “1st International HAV standard” (WHO, NIBSC 00/560) has been set up from 1 to nominal 0.000316 IU/μl and analysed by means of the *ABI PRISM 7000*, *7700* and *7900HT Sequence Detection Systems* with the help of the *artus* HAV TM RT-PCR Kit v2.0. 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 7000 SDS*) is shown in Fig. 29.

| Detection limit (p = 0.05) | |
|-----------------------------|-----------|
| <i>ABI PRISM 7000 SDS</i> | 0.2 IU/μl |
| <i>ABI PRISM 7700 SDS</i> | 0.1 IU/μl |
| <i>ABI PRISM 7900HT SDS</i> | 0.1 IU/μl |

This means that there is a 95 % probability that 0.2 IU/μl (*ABI PRISM 7000 SDS*), 0.1 IU/μl (*ABI PRISM 7700 SDS*) and 0.1 IU/μl (*ABI PRISM 7900HT SDS*) will be detected.

Probit analysis: Hepatitis A virus (ABI PRISM 7000 SDS)

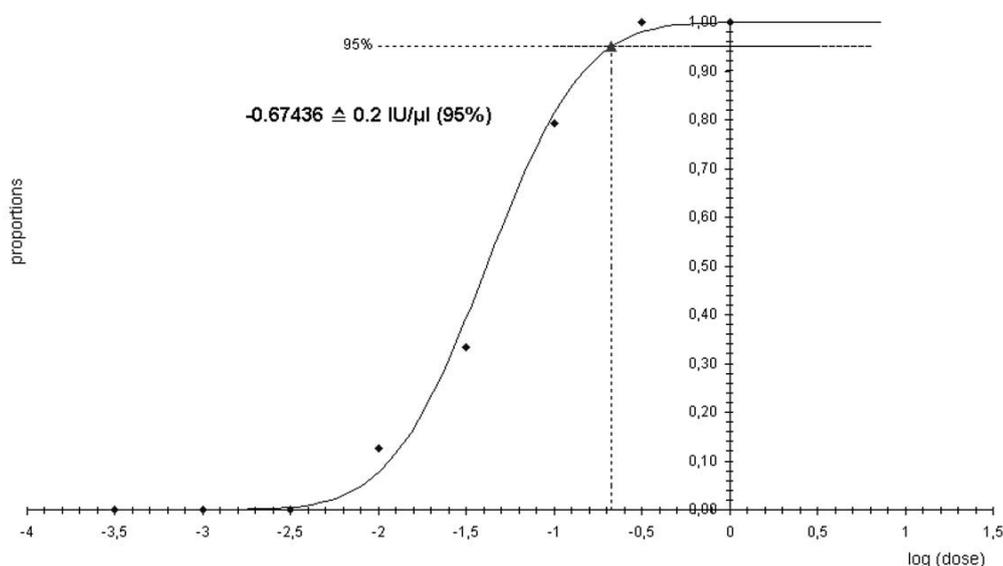


Fig. 29: Analytical sensitivity of the *artus* HAV TM RT-PCR Kit v2.0 (*ABI PRISM 7000 SDS*).

10.2 Specificity

The specificity of the *artus* HAV TM RT-PCR Kit v2.0 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 genotypes has thus been ensured by a database alignment and by a PCR run on an *ABI PRISM 7000 SDS* with the following genotypes (see Table 1):

Table 1: Testing of the specificity of relevant genotypes.

| Hepatitis A-virus strain | Genotypes | Source | HAV (FAM) | Internal Control (VIC) |
|--------------------------|---------------|------------------------------------|-----------|------------------------|
| HAV GBM | Genotype IA | Cell-culture material [¶] | + | + |
| HAV HM175 | Genotype IB | Cell-culture material [¶] | + | + |
| HAV 9F94 | Genotype II | Synthetic nucleic acid | + | + |
| HAV HMM | Genotype IIIA | Cell-culture material [¶] | + | + |
| HAV SLF-88 | Genotype VII | Synthetic nucleic acid | + | + |

[¶]Institute for Virology, University of Bremen, Germany

Moreover, the specificity was validated with 30 different HAV negative plasma samples. These did not generate any signals with the HAV specific primers and probes, which are included in the *HAV TM Master 1 & 2*.

To determine the specificity of the *artus* HAV TM RT-PCR Kit v2.0 the control group listed in the following table (see Table 2) has been tested for cross-reactivity. None of the tested pathogens has been reactive.

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

| Control group | HAV (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 5 (Cytomegalovirus) | - | + |
| Human immunodeficiency virus | - | + |
| Hepatitis B virus | - | + |
| Hepatitis C virus | - | + |
| Echovirus 6 | - | + |
| Echovirus 9 | - | + |
| Echovirus 11 | - | + |
| Echovirus 22 | - | + |
| Echovirus 24 | - | + |
| Rhinovirus | - | + |
| Poliovirus 2 | - | + |
| Enterovirus 71 | - | + |
| Coxsackievirus A9 | - | + |
| Coxsackievirus A16 | - | + |
| Coxsackievirus B5 | - | + |

10.3 Precision

The precision data of the *artus* HAV TM RT-PCR Kit v2.0 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* HAV TM RT-PCR Kit v2.0 have been collected using a 1 IU/μl dilution of the “1st International HAV standard” (WHO, NIBSC 00/560). Testing was performed with eight replicates. The precision data were calculated on basis of the Ct values of the amplification curves (Ct: threshold cycle, see Table 3). In addition, precision data for quantitative results in IU/μl were determined using the corresponding Ct values (see Table 4). Based on these results, the overall statistical spread of any given sample with the mentioned concentration is 4.77 % (Ct) or 57.11 % (conc.), for the detection of the *Internal Control* 2.62 % (Ct). These values are based on the totality of all single values of the determined variabilities.

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

| | Standard deviation | Variance | Coefficient of variation [%] |
|---|---------------------------|-----------------|-------------------------------------|
| Intra-assay variability: HAV Standard [‡] 1 IU/μl | 0.93 | 0.86 | 2.93 |
| Intra-assay variability: <i>Internal Control</i> | 0.68 | 0.46 | 2.57 |
| Inter-assay variability: HAV Standard [‡] 1 IU/μl | 1.23 | 1.51 | 3.77 |
| Inter-assay variability: <i>Internal Control</i> | 0.51 | 0.26 | 1.94 |
| Inter-batch variability: HAV Standard [‡] 1 IU/μl | 1.74 | 3.04 | 5.20 |
| Inter-batch variability: <i>Internal Control</i> | 0.84 | 0.71 | 3.18 |
| Total variance: HAV Standard [‡] 1 IU/μl | 1.57 | 2.46 | 4.77 |
| Total variance: <i>Internal Control</i> | 0.69 | 0.48 | 2.62 |

[‡]WHO 1st International HAV Standard (NIBSC 00/560)

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

| | Standard deviation | Variance | Coefficient of variation [%] |
|---|--------------------|----------|------------------------------|
| Intra-assay variability: HAV Standard ^m 1 IU/μl | 0.23 | 0.56 | 63.26 |
| Inter-assay variability: HAV Standard ^m 1 IU/μl | 0.63 | 0.40 | 65.42 |
| Inter-batch variability: HAV Standard ^m 1 IU/μl | 0.54 | 0.30 | 49.07 |
| Total variance: HAV Standard ^m 1 IU/μl | 0.61 | 0.37 | 57.11 |

^mWHO 1st International HAV Standard (NIBSC 00/560)

10.4 Robustness

The verification of the robustness allows the determination of the total failure rate of the *artus* HAV TM RT-PCR Kit v2.0. 30 HAV negative samples of plasma were spiked with 0.6 IU/μl elution volume of HAV control RNA (threefold concentration of the analytical sensitivity limit). After extraction using the QIAamp DSP Virus Kit (see **7.1 RNA Isolation**) these samples were analysed with the *artus* HAV TM RT-PCR Kit v2.0. For all HAV samples the failure rate was 0 %. In addition, the robustness of the *Internal Control* was assessed by purification and analysis of 30 HAV negative plasma samples. The total failure rate was 0 %. Inhibitions were not observed. Thus, the robustness of the *artus* HAV TM RT-PCR Kit v2.0 is ≥ 99 %.

11. Product Use Limitations

- The *artus* HAV TM RT-PCR Kit v2.0 is for research use only. Not for use in diagnostic procedures.
- No claim or representation is intended for their use for a specific clinical use (diagnostic, prognostic, or therapeutic). It is the user's responsibility to validate the performance of the *artus* HAV TM RT-PCR Kit v2.0 for any particular use.
- 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. Safety Information

For safety information of the *artus* HAV TM RT-PCR Kit v2.0, please consult the appropriate material safety data sheet (MSDS). The MSDS are available online in convenient and compact PDF format at www.qiagen.com/support/msds.aspx.

13. Quality Control

In accordance with QIAGEN's ISO 9001 and ISO 13485-certified Total Quality Management System, each lot of *artus* HAV TM RT-PCR Kit v2.0 is tested against predetermined specifications to ensure consistent product quality.

14. References

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

15. Explanation of Symbols



Use by



Batch code



Manufacturer



Catalogue number



Material number



Handbook



Components



Contains



Number



<N>

Contains sufficient for <N> tests



Temperature limitation



Consult instructions for use

QS

Quantitation Standard

IC

Internal Control

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artus HAV TM RT-PCR Kit v2.0

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