artus® Enterovirus LC RT-PCR Kit Handbook

Σ 24 (catalog no. 4510003)

Σ 96 (catalog no. 4510005)

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

For use with the *LightCycler*® Instrument

June 2007 – Version 1

REF 4510003, 4510005

HB 1046927

QIAGEN GmbH, QIAGEN Strasse 1, D-40724 Hilden

R1 MAT 1046927

QIAGEN®

Sample & Assay Technologies

artus Enterovirus LC RT-PCR Kit

Trademarks and Disclaimers
QIAGEN®, QIAamp®, *artus*®, (QIAGEN Group), *LightCycler*® (Roche Diagnostics)

Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

The *artus* Enterovirus LC RT-PCR Kit is for research use only. Not for use in diagnostic procedures.

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.

Table of Contents

artus® Enterovirus LC RT-PCR Kit

For use with the *LightCycler*® Instrument.

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

1. Contents

	Labelling and contents	Art. No. 4510003 24 reactions	Art. No. 4510005 96 reactions
Blue	*Enterovirus LC Master*	2 x 12 rxns	8 x 12 rxns
Red	*Enterovirus LC QS 1*¤ *6 x 10^4 cop/µl*	1 x 200 µl	1 x 200 µl
Red	*Enterovirus LC QS 2*¤ *6 x 10^3 cop/µl*	1 x 200 µl	1 x 200 µl
Red	*Enterovirus LC QS 3*¤ *6 x 10^2 cop/µl*	1 x 200 µl	1 x 200 µl
Red	*Enterovirus LC QS 4*¤ *6 x 10^1 cop/µl*	1 x 200 µl	1 x 200 µl
Green	*Enterovirus LC IC*¤	1 x 1,000 µl	2 x 1,000 µl
White	*Water* (*PCR grade*)	1 x 1,000 µl	1 x 1,000 µl

¤ *QS* = *Quantitation Standard*
IC = *Internal Control*

2. Storage

The components of the *artus* Enterovirus LC RT-PCR Kit 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 **8.1 RNA Isolation**)
- Pipettes (adjustable)
- Sterile pipette tips with filters
- Vortex mixer
- Desktop centrifuge with rotor for 2 ml reaction tubes
- *Color Compensation Set* (Roche Diagnostics, Cat. No. 2 158 850) for the installation of a *Crosstalk Color Compensation* file
- *LightCycler*® Capillaries (20 µl)
- *LightCycler*® Cooling Block
- *LightCycler*® Instrument
- *LightCycler*® Capping Tool

4. General Precautions

The user should always pay attention to the following:

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

5. Pathogen Information

Enteroviruses belong to the family *picornaviridae* including over 70 distinct serotypes (coxsackie A and B, echoviruses, polioviruses and enteroviruses 68 - 71). They infect a wide range of mammals and are associated with a broad spectrum of diseases. There are 68 viruses within the Enterovirus genus that are known to infect humans. Enteroviruses are transmitted

primarily by the fecal-oral route, but also respiratory spread is possible with some of the Coxsackieviruses, which can cause infections of the upper respiratory tract. Non-polio enteroviruses most commonly cause rashes, upper respiratory tract infections (URTIs) and summer colds. Furthermore, Enterovirus infections account for a substantial number of aseptic meningitis and encephalitis patients requiring hospitalization in summer and fall.

6. 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).

7. Product Description

The *artus* Enterovirus LC RT-PCR Kit constitutes a ready-to-use system for the detection of Enterovirus RNA using polymerase chain reaction (PCR) in the *LightCycler*® Instrument. The *Enterovirus LC Master* contains reagents and enzymes for the reverse transcription and specific amplification of a 114 bp region of the Enterovirus genome, and for the direct detection of the specific amplicon in fluorimeter channel F1 of the *LightCycler*® Instrument. In addition, the *artus* Enterovirus LC RT-PCR Kit contains a second heterologous amplification system to identify possible PCR inhibition. This is detected as an *Internal Control* (*IC*) in fluorimeter channel F3. The detection limit of the analytical Enterovirus RT-PCR (see **11.1 Analytical Sensitivity**) is not reduced. External positive controls (*Enterovirus LC 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 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 kit is recommended:

Sample Material	Nucleic Acid Isolation Kit	Catalogue Number	Manufacturer	Carrier RNA
CSF	QIAamp Viral RNA Mini Kit (50)	52 904	QIAGEN	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, we recommend the following procedure deviant from the user manual of the extraction kit:
  a. Resuspend the lyophilised carrier RNA prior to first use of the extraction kit in 310 µl of the elution buffer provided with the kit (final concentration 1 µg/µl, do not use lysis buffer). Portion this carrier RNA solution into a number of aliquots adequate to your needs and store them at -20°C. Avoid repeated thawing (> 2 x) of a carrier RNA aliquot.
  b. Before the beginning of each extraction, a mixture of lysis buffer and carrier RNA (and *Internal Control*, where applicable, see **8.2 Internal Control**) should be prepared freshly according to the following pipetting scheme:

Number of samples	1	12
Lysis buffer 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**

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

- 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* Enterovirus LC RT-PCR Kit should not be used with **phenol**-based isolation methods.

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

8.2 Internal Control

An *Internal Control* (*Enterovirus LC 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 QIAmp Viral RNA Mini 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 **8.1 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 0.5 µl of the *Internal Control* per reaction directly to 15 µl *Enterovirus LC Master*. For each

PCR reaction use 15 µl of the Master Mix produced as described above* and add 5 µl of the purified sample. If you are preparing a PCR run for several samples please increase the volume of the *Enterovirus LC Master* and the *Internal Control* according to the number of samples (see **8.4 Preparing the PCR**).

8.3 Quantitation

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

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

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

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

* 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.

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

8.4 Preparing the PCR

Make sure that the Cooling Block as well as the capillary adapters (accessories of the *LightCycler*® Instrument) are pre-cooled to +4°C. Place the desired number of *LightCycler*® capillaries into the adapters of the Cooling Block. Please make sure that at least one *Quantitation Standard* as well as one negative control (*Water*, *PCR grade*) are included per PCR run. To generate a standard curve, use all supplied *Quantitation Standards* (*Enterovirus LC 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 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 **8.2 Internal Control**). In this case, please use the following pipetting scheme (for a schematic overview see Fig. 1):

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

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

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

Pipette 15 µl of the Master Mix into the plastic reservoir of each capillary. Then add 5 µl of the eluted sample RNA. Correspondingly, 5 µl of at least one of the *Quantitation Standards* (*Enterovirus LC QS 1 - 4*) must be used as a positive control and 5 µl of water (*Water*, *PCR grade*) as a negative control. Close the capillaries. To transfer the mixture from the plastic reservoir into the capillary, centrifuge the adapters containing the capillaries in a desktop centrifuge for ten seconds at a maximum of 400 x g (2,000 rpm).

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

Addition of the *Internal Control* to the Purification Procedure

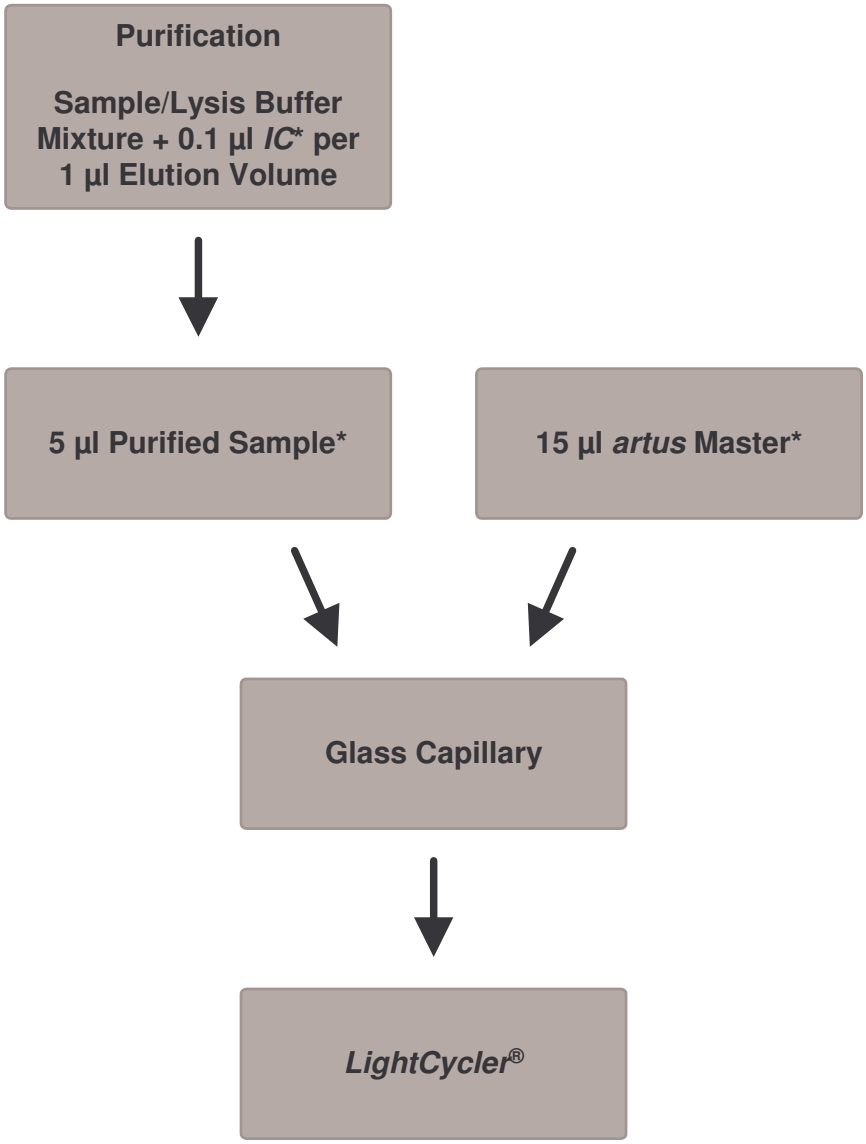

Fig. 1: Schematic workflow for the control of both the purification procedure and PCR inhibition.

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

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

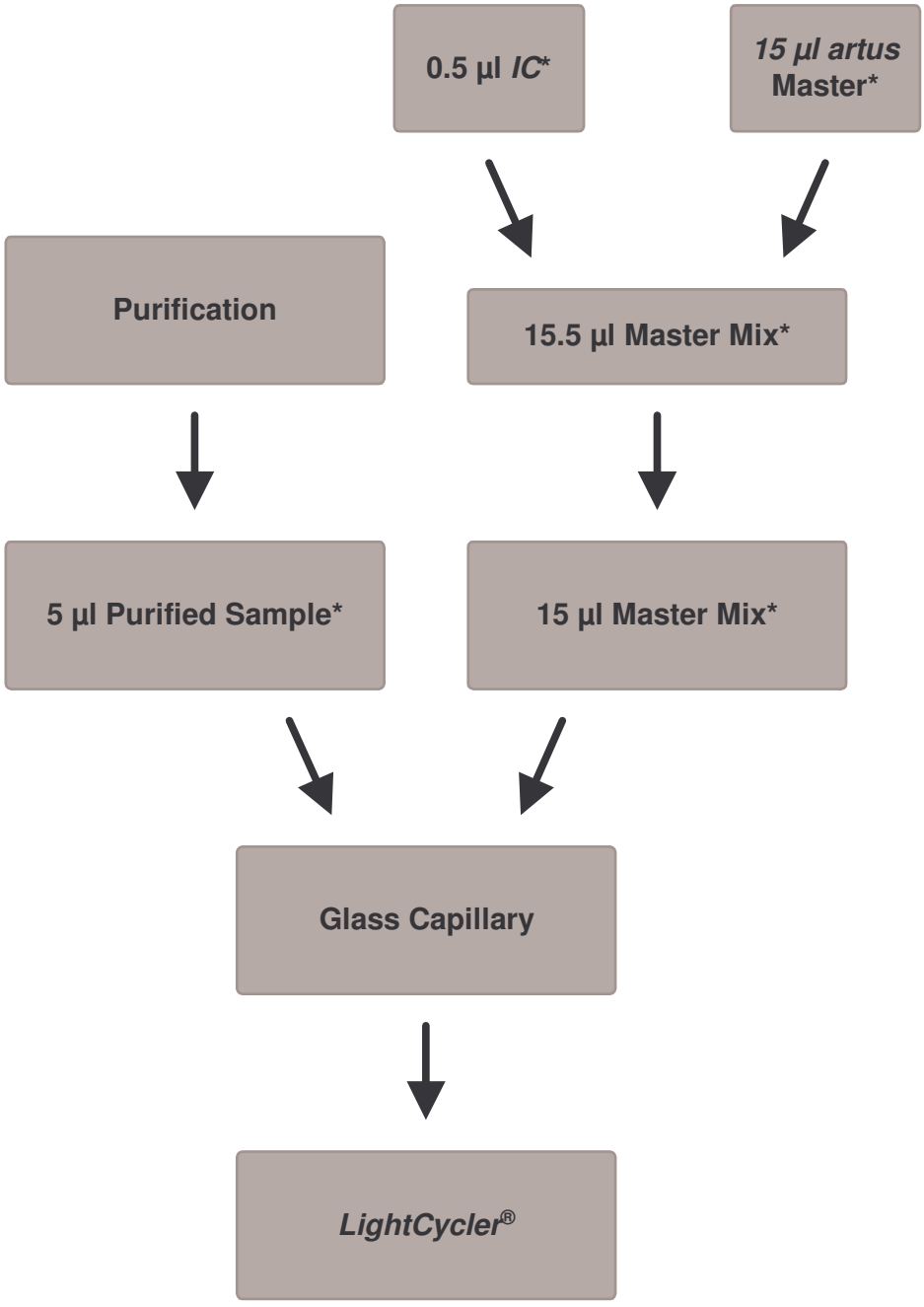

Fig. 2: Schematic workflow for the control of PCR inhibition.

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

8.5 Programming of the *LightCycler*® Instrument

For the detection of Enterovirus RNA, create a temperature profile on your *LightCycler*® Instrument according to the following four steps (see Fig. 3 - 6).

A.	Reverse Transcription of the RNA	Fig. 3
B.	Initial Activation of the Hot Start Enzyme	Fig. 4
C.	Amplification of the cDNA	Fig. 5
D.	Cooling	Fig. 6

Pay particular attention to the settings for *Analysis Mode*, *Cycle Program Data* and *Temperature Targets*. In the illustrations these settings are framed in bold black. Please find further information on programming the *LightCycler*® Instrument in the *LightCycler Operator's Manual*.

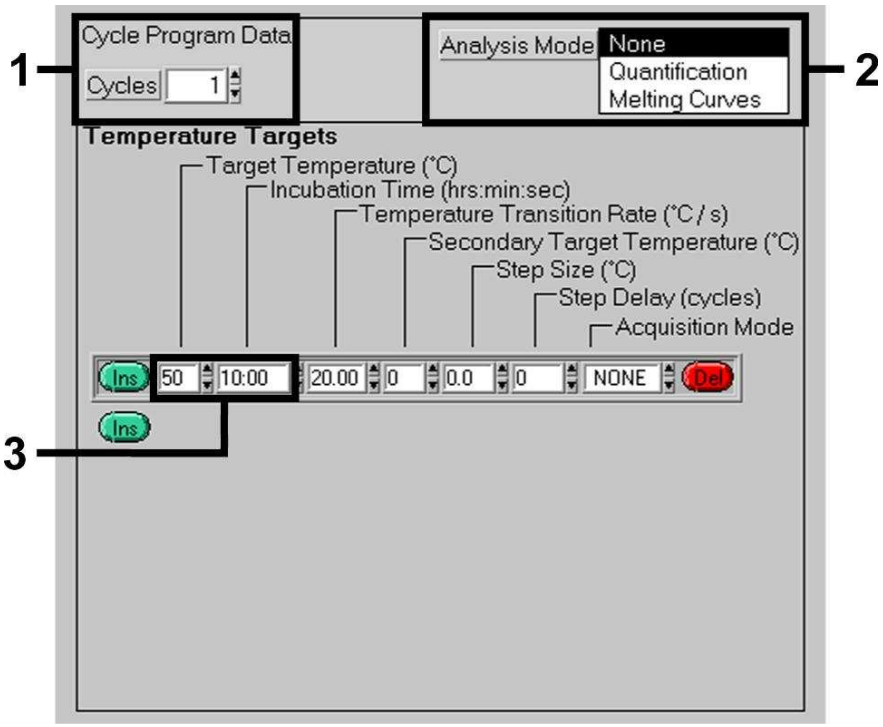

Fig. 3: Reverse Transcription of the RNA.

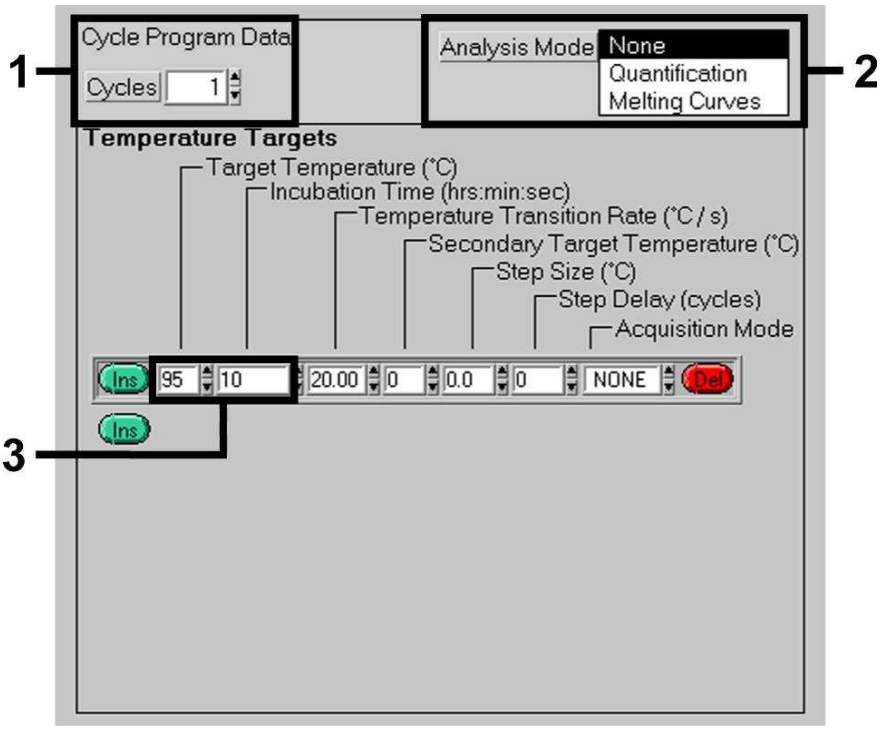

Fig. 4: Initial Activation of the Hot Start Enzyme.

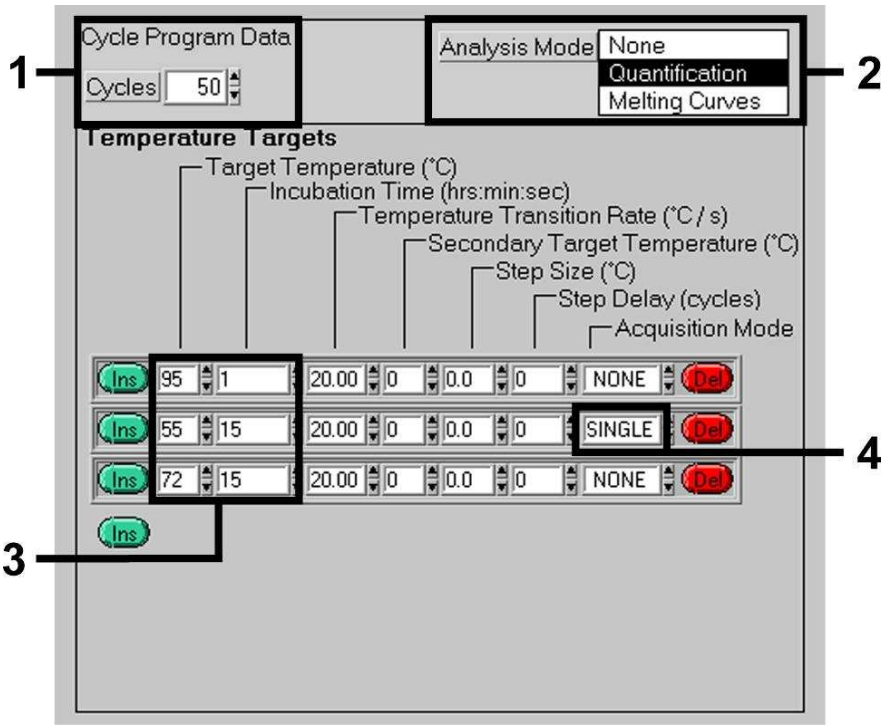

Fig. 5: Amplification of the cDNA.

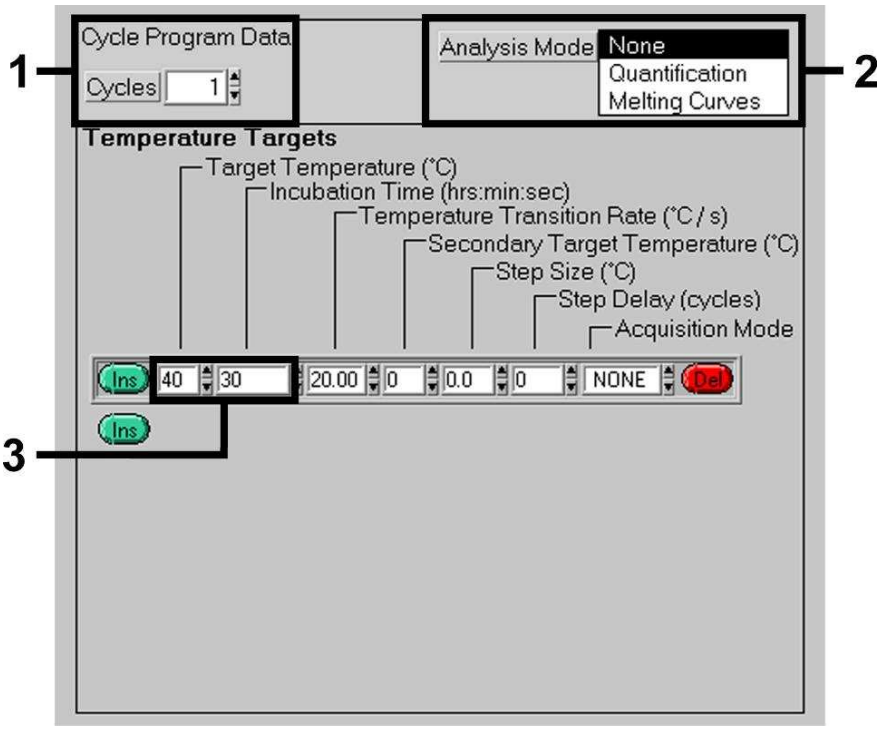

Fig. 6: Cooling.

9. Data Analysis

In multicolour analyses interferences occur between fluorimeter channels. The *LightCycler*® Instrument's software contains a file termed *Color Compensation File*, which compensates for these interferences. Open this file before, during or after the PCR run by activating the *Choose CCC File* or the *Select CC Data* button. If no *Color Compensation File* is installed, generate the file according to the instructions in the *LightCycler Operator's Manual*. After the *Color Compensation File* has been activated, separate signals appear in fluorimeter channels F1, F2 and F3. For analysis of the PCR results gained with the *artus* Enterovirus LC RT-PCR Kit please select fluorescence display options F1 for the analytical Enterovirus RT-PCR and F3/Back-F1 for the *Internal Control* RT-PCR, respectively. For the analysis of quantitative runs, please follow the instructions given in **8.3 Quantitation** and in the **Technical Note for quantitation on the *LightCycler*® *1.1/1.2/1.5* or *LightCycler*® *2.0* Instrument** at **www.qiagen.com/Products/ByLabFocus/MDX**.

The following results are possible:

1. A signal is detected in fluorimeter channel F1.

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

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

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

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

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

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

   No result 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. 7 and Fig. 8.

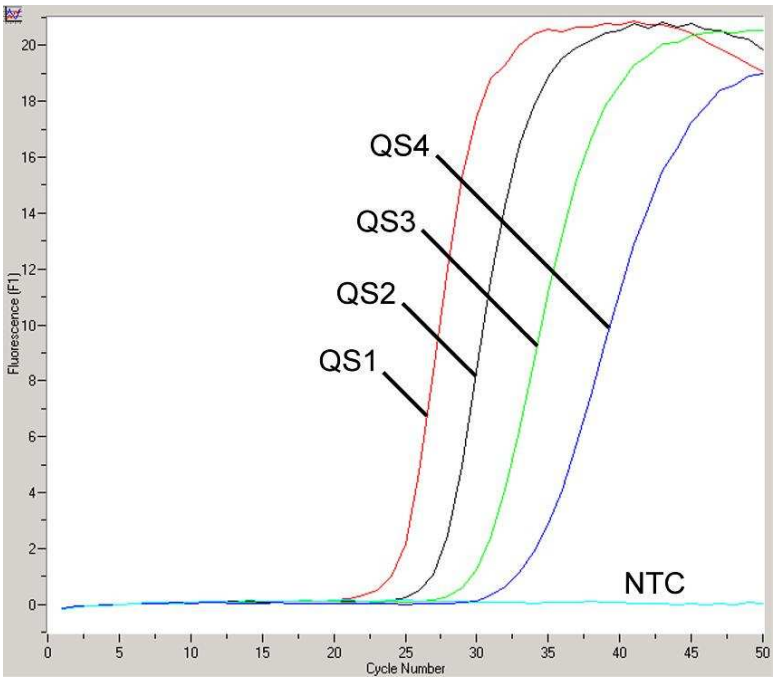

Fig. 7: Detection of the *Quantitation Standards* (*Enterovirus LC QS 1 - 4*) in fluorimeter channel F1. NTC: non-template control (negative control).

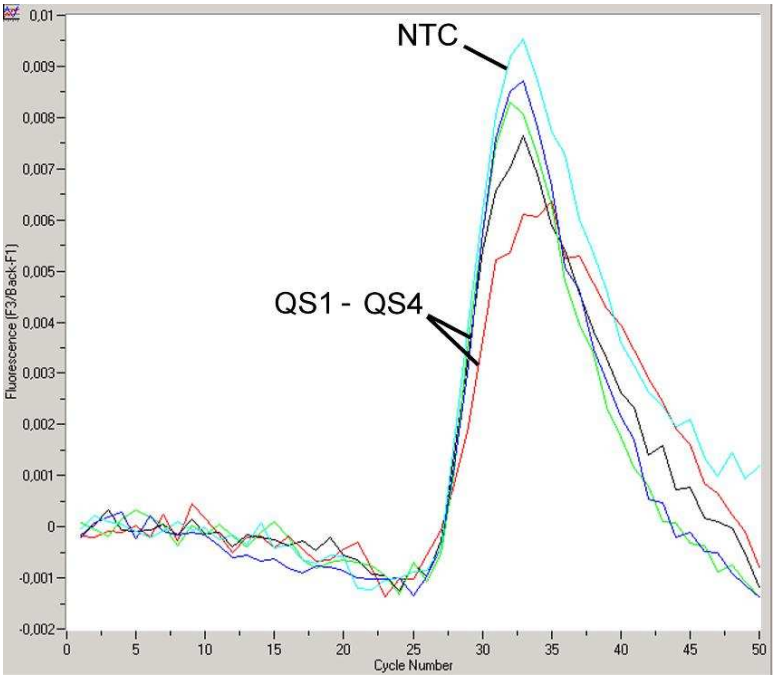

Fig. 8: Detection of the *Internal Control* (*IC*) in fluorimeter channel F3/Back-F1 with simultaneous amplification of *Quantitation Standards* (*Enterovirus LC QS 1 - 4*). NTC: non-template control (negative control).

10. Troubleshooting

No signal with positive controls (*Enterovirus LC QS 1 - 4*) in fluorimeter channel F1:

- The selected fluorimeter channel for PCR data analysis does not comply with the protocol.
  - → For data analysis select the fluorimeter channel F1 for the analytical Enterovirus RT-PCR and the fluorimeter channel F3/Back-F1 for the *Internal Control* RT-PCR.
- Incorrect programming of the temperature profile of the *LightCycler*® Instrument.
  - → Compare the temperature profile with the protocol (see **8.5 Programming of the *LightCycler*® Instrument**).
- Incorrect configuration of the PCR reaction.
  - → Check your work steps by means of the pipetting scheme (see **8.4 Preparing the PCR**) and repeat the PCR, if necessary.
- The storage conditions for one or more kit components did not comply with the instructions given in **2. Storage** or the *artus* Enterovirus LC RT-PCR Kit had expired.
  - → Please check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.

Weak or no signal of the *Internal Control* in fluorimeter channel F3/Back-F1 and simultaneous absence of a signal in channel F1:

- The PCR conditions do not comply with the protocol.
  - → Check the PCR conditions (see above) and repeat the PCR with corrected settings, if necessary.
- The PCR was inhibited.
  - → Make sure that you use a recommended isolation method (see **8.1 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 **8.1 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 **8.1 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* Enterovirus LC RT-PCR Kit had expired.
  - ➔ Please check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.

Signals with the negative controls in fluorimeter channel F1 of the analytical RT-PCR.

- A contamination occurred during preparation of the PCR.
  - ➔ Repeat the PCR with new reagents in replicates.
  - ➔ If possible, close the PCR tubes directly after addition of the sample to be tested.
  - ➔ Strictly pipette the positive controls at last.
  - ➔ Make sure that work space and instruments are decontaminated at regular intervals.
- A contamination occurred during extraction.
  - ➔ Repeat the extraction and PCR of the sample to be tested using new reagents.
  - ➔ Make sure that work space and instruments are decontaminated at regular intervals.

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

11. Specifications

11.1 Analytical Sensitivity

In order to determine the analytical sensitivity of the *artus* Enterovirus LC RT-PCR Kit, a standard dilution series has been set up from 60 to nominal 0.02 of *in vitro* transcribed RNA copies per µl of the Enterovirus amplicon and analysed with the *artus* Enterovirus LC RT-PCR Kit. Testing was carried out on three different days on eight replicates. The results were determined by a probit analysis. A graphical illustration of the probit analysis is shown in Fig. 9. The analytical detection limit of the *artus* Enterovirus LC RT-PCR Kit is consistently 3.2 copies/µl (p = 0.05). This means that there is a 95 % probability that 3.2 copies/µl will be detected.

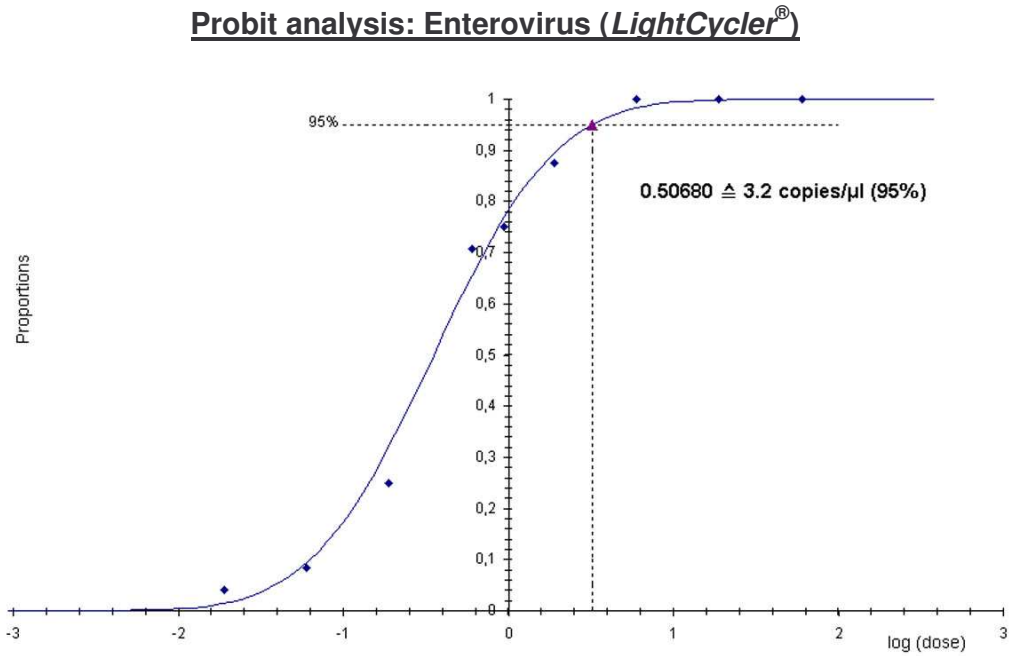

Fig. 9: Analytical sensitivity of the *artus* Enterovirus LC RT-PCR Kit.

11.2 Specificity

The specificity of the *artus* Enterovirus LC RT-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 enteroviruses has thus been ensured. Moreover, the specificity was validated with 100 different Enterovirus negative samples.

If other sample material than CSF should be used for the test, cross reactions with Rhinovirus 1b may appear.

12. Product Use Limitations

- The *artus* Enterovirus LC RT-PCR Kit 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* Enterovirus LC RT-PCR Kit 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.

13. Safety Information

For safety information of the *artus* Enterovirus LC RT-PCR Kit, 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**.

14. Quality Control

In accordance with QIAGEN's ISO 9001 and ISO 13485-certified Total Quality Management System, each lot of *artus* Enterovirus LC RT-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

<N>

Contains sufficient for <N> tests

Temperature limitation

QS *Quantitation Standard*

IC *Internal Control*

Austria ■ **QIAGEN Vertriebs GmbH** ■ Löwengasse 47/6 ■ 1030 Wien
Orders 0800/28-10-10 ■ Fax 0800/28-10-19 ■ Technical 0800/28-10-11

Canada ■ **QIAGEN Inc.** ■ 2800 Argentia Road ■ Unit 7 ■ Mississauga ■ Ontario ■ L5N 8L2
Orders 800-572-9613 ■ Fax 800-713-5951 ■ Technical 800-DNA-PREP (800-362-7737)

France ■ **QIAGEN S.A.** ■ 3 avenue du Canada ■ LP 809 ■ 91974 COURTABOEUF CEDEX
Orders 01-60-920-920 ■ Fax 01-60-920-925 ■ Technical 01-60-920-930

Germany ■ **QIAGEN GmbH** ■ QIAGEN Strasse 1 ■ 40724 Hilden
Orders 02103-29-12000 ■ Fax 02103-29-22000 ■ Technical 02103-29-12400

Italy ■ **QIAGEN S.p.A**. ■ Via Grosio, 10/10 ■ 20151 Milano
Orders 02-33430-411 ■ Fax 02-33430-426 ■ Technical 800-787980

Japan ■ **QIAGEN K.K.** ■ Forefront Tower II ■ 13-1, Kachidoki 3 Chome ■ Chuo-ku, Tokyo 104-0054
Telephone 03-5547-0811 ■ Fax 03-5547-0818 ■ Technical 03-5547-0811

Switzerland ■ **QIAGEN AG** ■ Garstligweg 8 ■ 8634 Hombrechtikon
Orders 055-254-22-11 ■ Fax 055-254-22-13 ■ Technical 055-254-22-12

USA ■ **QIAGEN Inc.** ■ 27220 Turnberry Lane ■ Valencia ■ CA 91355
Orders 800-426-8157 ■ Fax 800-718-2056 ■ Technical 800-DNA-PREP (800-362-7737)

1046927

QIAGEN®

Sample & Assay Technologies