artus® Influenza/H5 LC RT-PCR Kit Handbook

Σ 24 (catalog no. 4522003)

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

For use with the *LightCycler*® Instrument

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QIAGEN®

Sample & Assay Technologies

artus Influenza/H5 LC RT-PCR Kit

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artus® Influenza/H5 LC RT-PCR Kit

For use with the *LightCycler*® Instrument.

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1. Contents

	Labelling and contents	Art. No. 4522003 24 reactions
Blue	*Influenza LC Master 1*	2 x 12 rxns
Black	*Influenza LC Master 2*	2 x 12 rxns
Violet	*Influenza/H5 LC Master*	2 x 12 rxns
Yellow	*Influenza LC Mg-Sol*¤	1 x 800 µl
Red	*Influenza LC Control*	1 x 200 µl
Brown	*Influenza/H5 LC Control*	1 x 200 µl
Green	*Influenza LC IC*¤	1 x 1,000 µl
White	*Water* (*PCR grade*)	1 x 1,000 µl

¤ *IC* = *Internal Control*
Mg-Sol = *Magnesium Solution*

2. Storage

The components of the *artus* Influenza/H5 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

Influenza (flu) remains one of the most crucial health problems throughout the world. Regularly, the human population gets hit by influenza pandemics with thousands of influenza deaths. The influenza virus gets spread via aerosols. An infection with influenza A or influenza B virus leads to severe infection predominantly of the respiratory tract, which can be fatal. An infection with influenza C virus normally causes a milder etiopathology.

The persistence of the influenza virus in the human population is due to its genetic material and its antigenic composition. The major surface antigens are hemagglutinin (H) and neuraminidase (N). The surface antigens change continuously (antigenic drift).

The *artus* Influenza/H5 LC RT-PCR Kit contains as an optional feature, an influenza/H5 specific detection reagent. This detection reagent contains primers and probes for the specific detection of hemagglutinin 5 (H5) sequences in sample material already tested positive with the influenza LC screening system. The primers and probes are designed to detect all currently known influenza/H5 sequences. In animals (birds) also other H5Nx subtype combinations should be considered.

Due to the fact that influenza viruses are rapidly evolving and especially the H5N1 strain is currently adapting to the human host, there is an increased risk that additional mutations occur which may not be detected with the influenza/H5 detection reagents.

Please always bear in mind that the molecular detection of influenza RNA in a sample is a strong indicator for an ongoing infection but a negative influenza RT-PCR result does not rule out viral replication.

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* Influenza/H5 LC RT-PCR Kit contains two ready-to-use systems for the detection of influenza virus specific RNA and influenza virus H5 RNA using polymerase chain reaction (PCR) in the *LightCycler*® Instrument. The ***Influenza LC Master 1*** and ***2*** contains reagents and enzymes for the reverse transcription and specific amplification of a 143 bp region of the influenza virus (A/B) genome, and for the direct detection of the specific amplicon in fluorimeter channel F1 of the *LightCycler*® Instrument. In addition, the *artus* Influenza/H5 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/Back-F1. The detection limit of the analytical influenza RT-PCR is not reduced. The ***Influenza/H5 LC Master*** contains reagents and enzymes for the reverse transcription and specific amplification of a 121 bp region of the influenza virus H5 genome, and for the direct detection of the specific amplicon in fluorimeter channel F2/Back-F1 of the *LightCycler*® Instrument. Two external positive controls (*Influenza LC Control* & *Influenza/H5 LC Control*)* are supplied.

Important: The influenza/H5 detection reagents do not contain an *Internal Control*. The influenza/H5 Master Mix may only be used after running the influenza RT-PCR.

* Contains in vitro transcribed subtype A RNA.

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
Sputum; throat and nasal swabs in viral transport medium	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* Influenza/H5 LC RT-PCR Kit should not be used with **phenol**-based isolation methods.

Important: The *Internal Control* of the *artus* Influenza/H5 LC RT-PCR Kit can be used directly in the isolation procedure (see **8.2 Internal Control**).

8.2 Internal Control

An *Internal Control* (*Influenza 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 QIAamp 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* and 3 µl *Influenza LC Mg-Sol* per reaction directly to 12 µl *Influenza LC Master 1/2*. 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 *Influenza LC Master 1/2*, the *Influenza LC Mg-Sol* and the *Internal Control* according to the number of samples (see **8.3 Preparing the PCR**).

Important: The influenza/H5 detection reagents do not contain an *Internal Control*. The influenza/H5 Master Mix may only be used after running the influenza RT-PCR.

8.3 Preparing the PCR

8.3.1 Preparing the Influenza 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 one positive control as well as one negative control (*Water*, *PCR grade*) are included per 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):

* 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.

	Number of samples	1	12
1. Preparation of Master Mix	*Influenza LC Master 1*	2 µl	24 µl
	Influenza LC Master 2	10 µl	120 µl
	Influenza LC Mg-Sol	3 µl	36 µl
	Influenza 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 *Influenza LC 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	*Influenza LC Master 1*	2 µl	24 µl
	Influenza LC Master 2	10 µl	120 µl
	Influenza LC Mg-Sol	3 µl	36 µl
	Influenza 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 to each tube. Correspondingly, 5 µl of *Influenza LC Control* 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.

Influenza PCR:
Addition of the *Internal Control* to the Purification Procedure

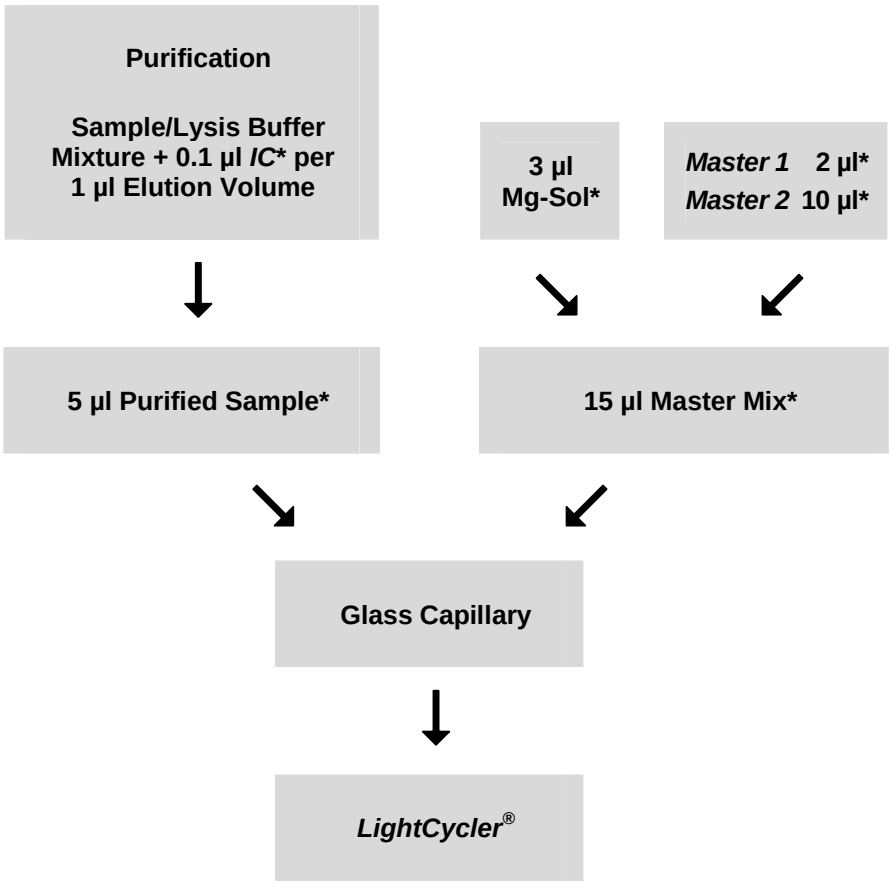

Fig. 1: Schematic workflow for the control of both the purification procedure and PCR inhibition.

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

Influenza PCR:
Addition of the *Internal Control* into the *artus* Master

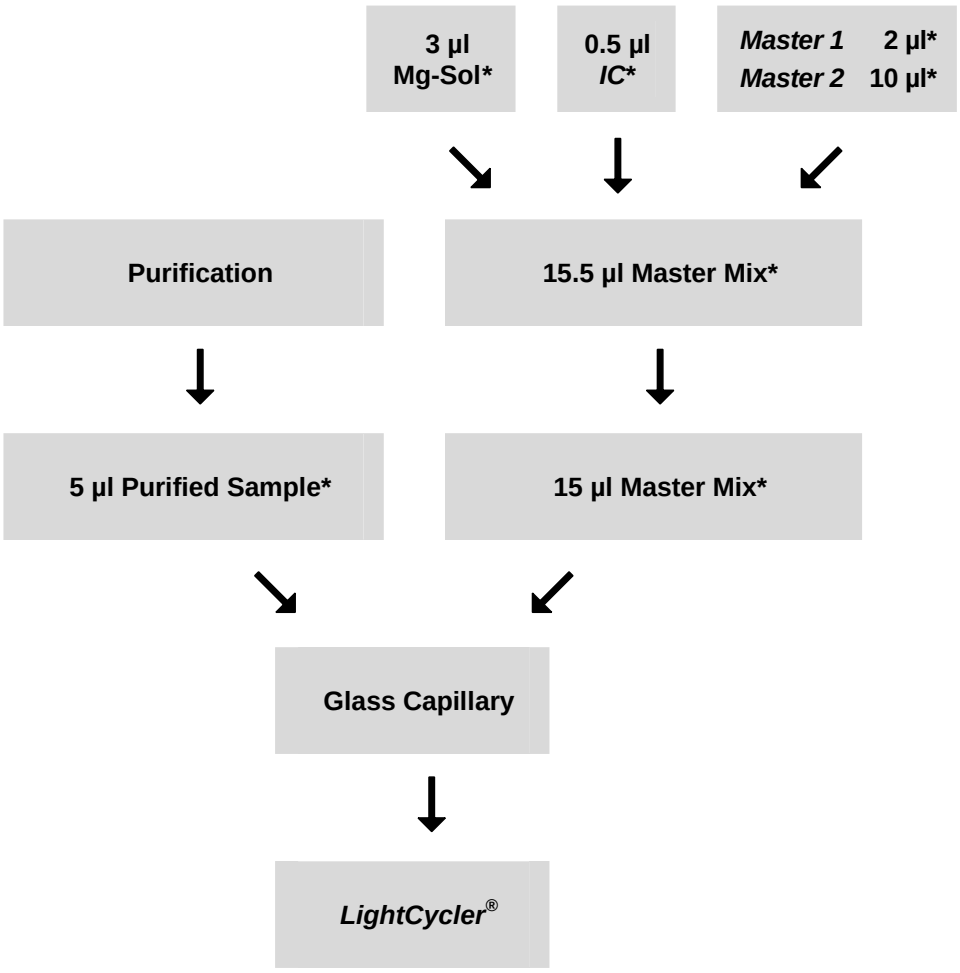

Fig. 2: Schematic workflow for the control of PCR inhibition.

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

8.3.2 Preparing the Influenza/H5 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 one positive control as well as one negative control (*Water*, *PCR grade*) are included per 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. For the preparation of the PCR assay please use the following pipetting scheme (for a schematic overview see Fig. 3):

	Number of samples	**1**	**12**
1. Preparation of Master Mix	*Influenza/H5 LC Master*	12 µl	144 µl
	Influenza LC Mg-Sol	3 µl	36 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**

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 *Influenza/H5 LC Control* must be used as a positive control and 5 µl of PCR grade water (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).

Important: The influenza/H5 detection reagents do not contain an *Internal Control*. The influenza/H5 Master Mix may only be used after running the influenza RT-PCR.

Preparing the Influenza/H5 PCR

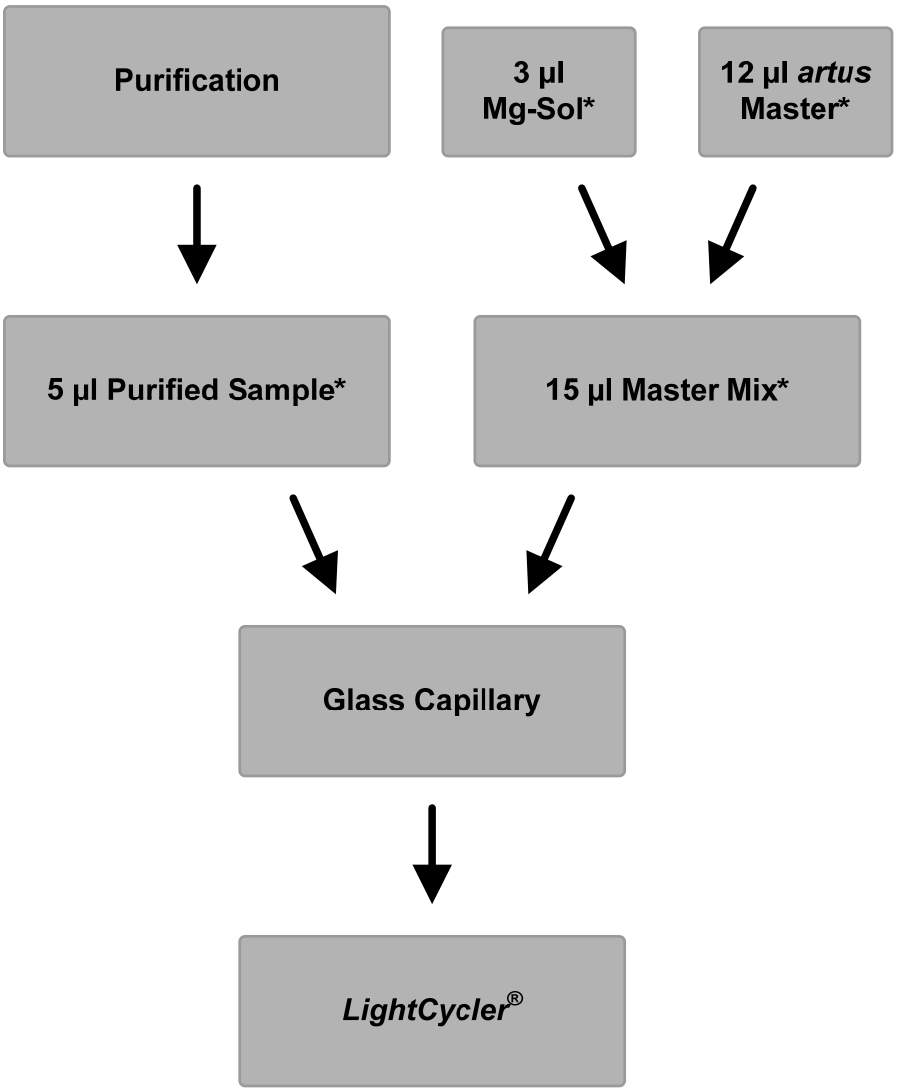

Fig. 3: Schematic workflow.

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

8.4 Programming of the *LightCycler*® Instrument

8.4.1 Programming of the Influenza PCR

For the detection of influenza virus RNA, create a temperature profile on your *LightCycler*® Instrument according to the following four steps (see Fig. 4 - 7).

A.	Reverse Transcription of the RNA	Fig. 4
B.	Initial Activation of the Hot Start Enzyme	Fig. 5
C.	Amplification of the cDNA	Fig. 6
D.	Cooling	Fig. 7

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

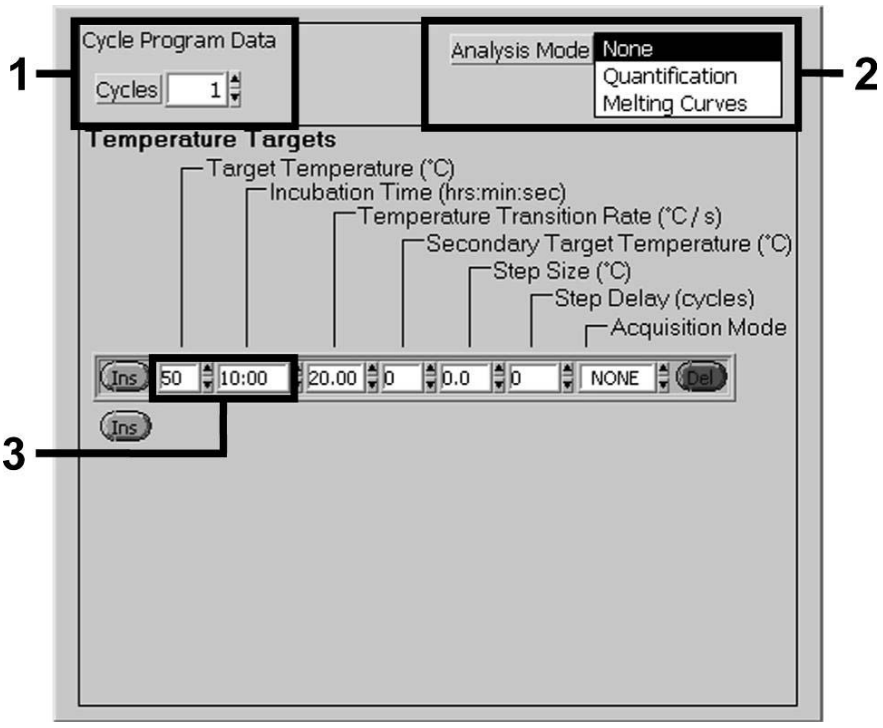

Fig. 4: Reverse Transcription of the RNA.

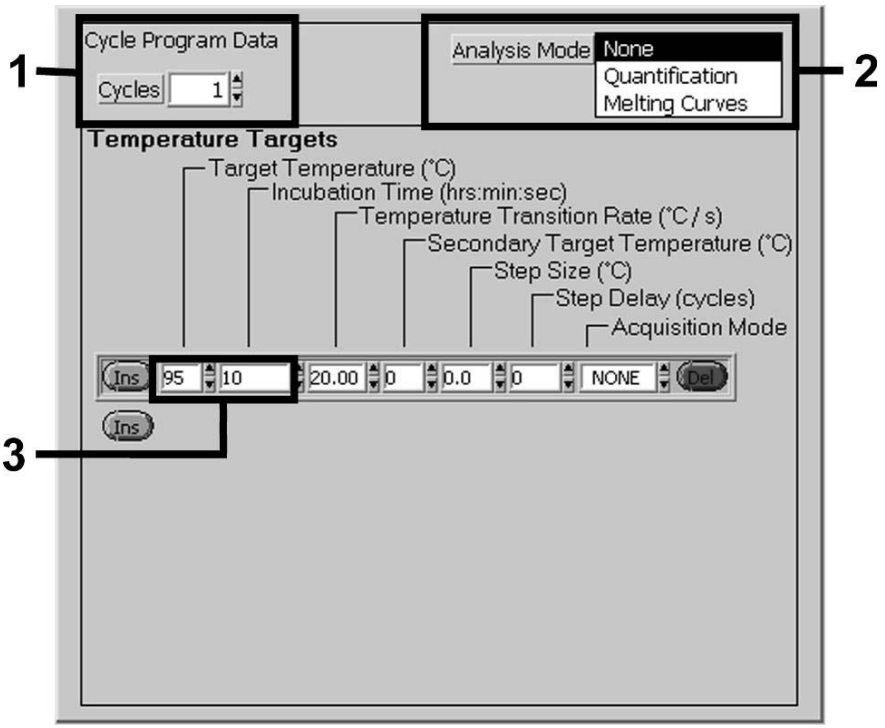

Fig. 5: Initial Activation of the Hot Start Enzyme.

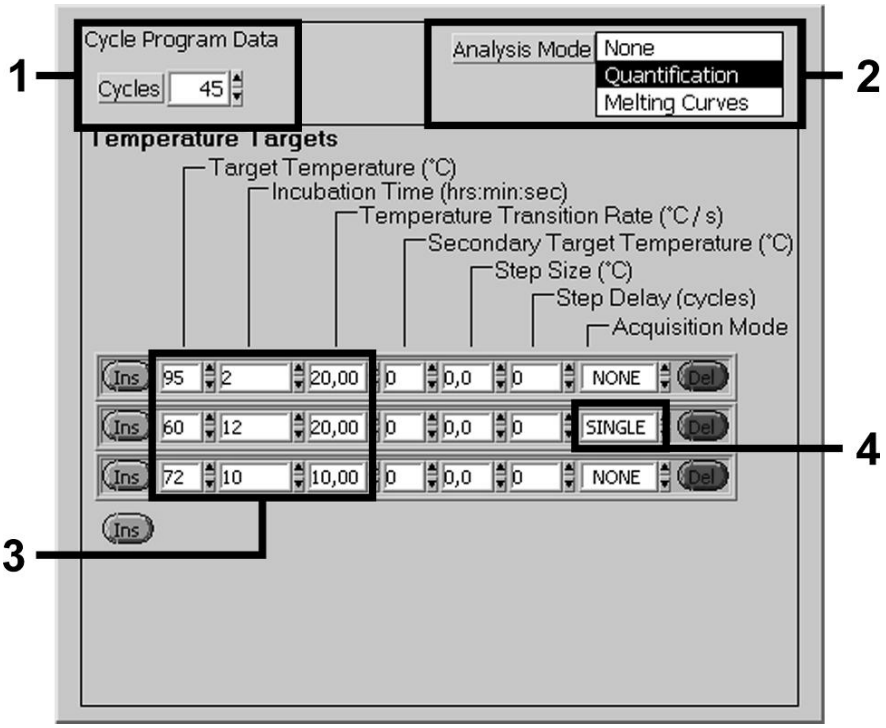

Fig. 6: Amplification of the cDNA.

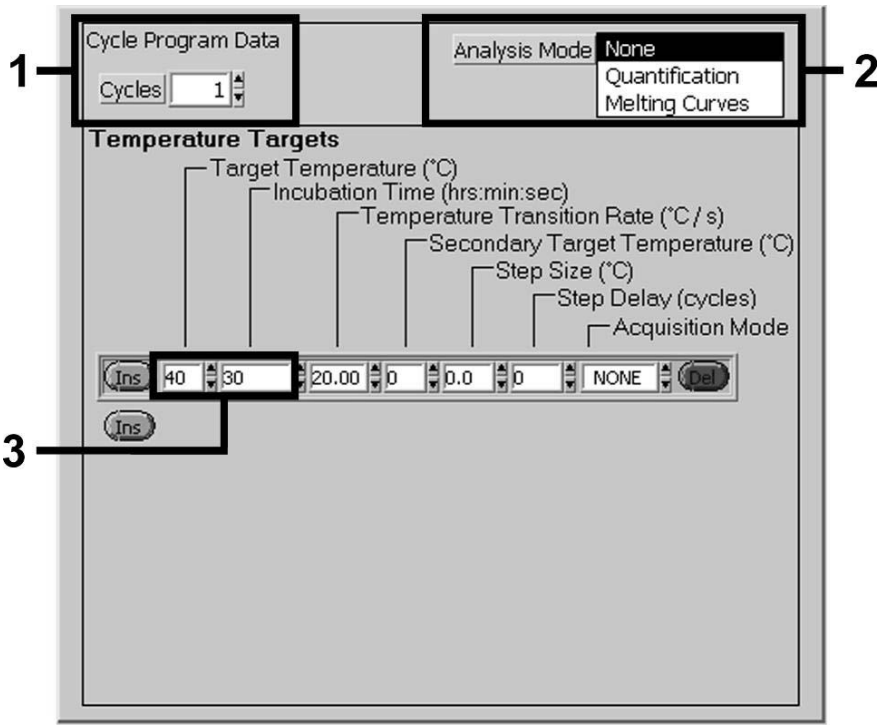

Fig. 7: Cooling.

8.4.2 Programming of the Influenza/H5 PCR

For the detection of influenza virus H5 RNA, create a temperature profile on your *LightCycler*® Instrument according to the following four steps (see Fig. 8 - 11).

A.	Reverse Transcription of the RNA	Fig. 8
B.	Initial Activation of the Hot Start Enzyme	Fig. 9
C.	Amplification of the cDNA	Fig. 10
D.	Cooling	Fig. 11

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*.

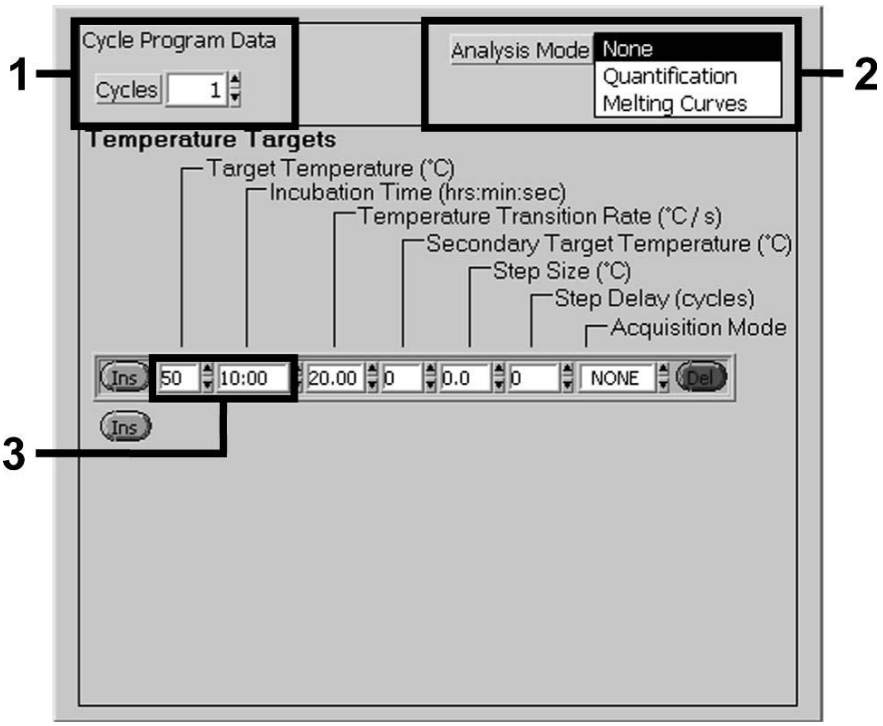

Fig. 8: Reverse Transcription of the RNA.

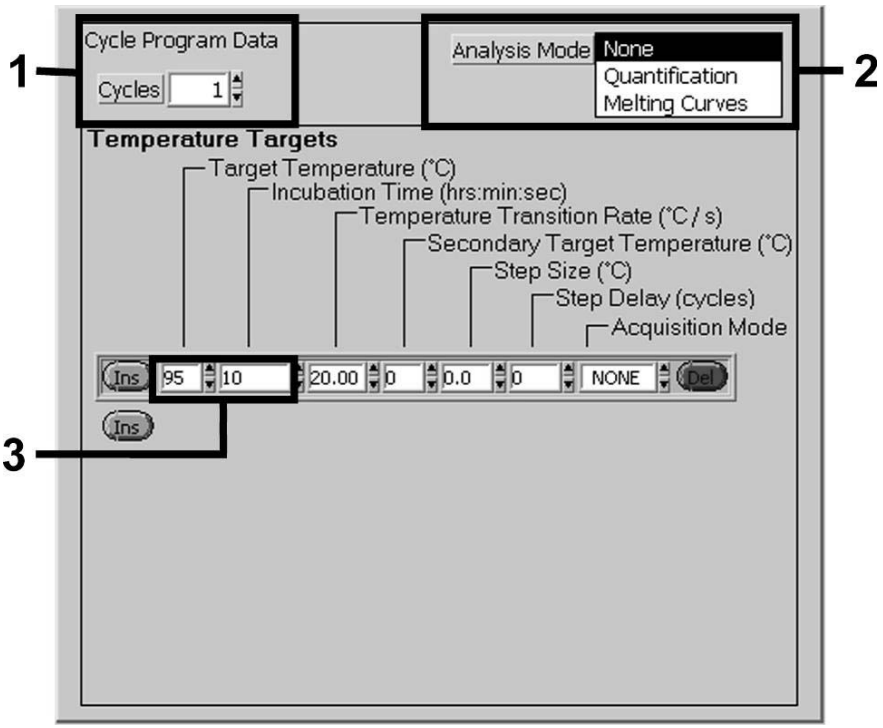

Fig. 9: Initial Activation of the Hot Start Enzyme.

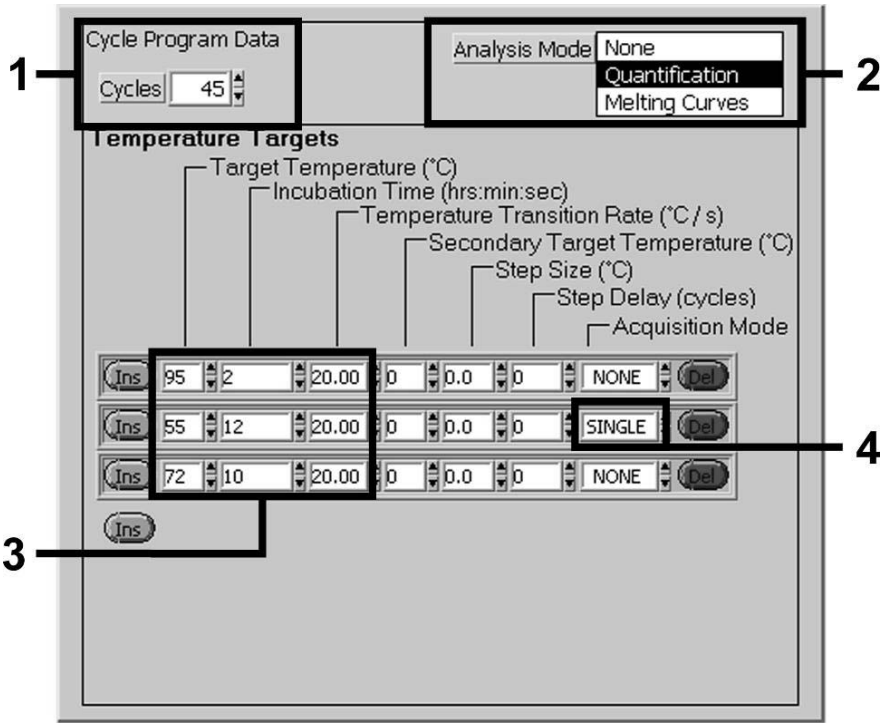

Fig. 10: Amplification of the cDNA.

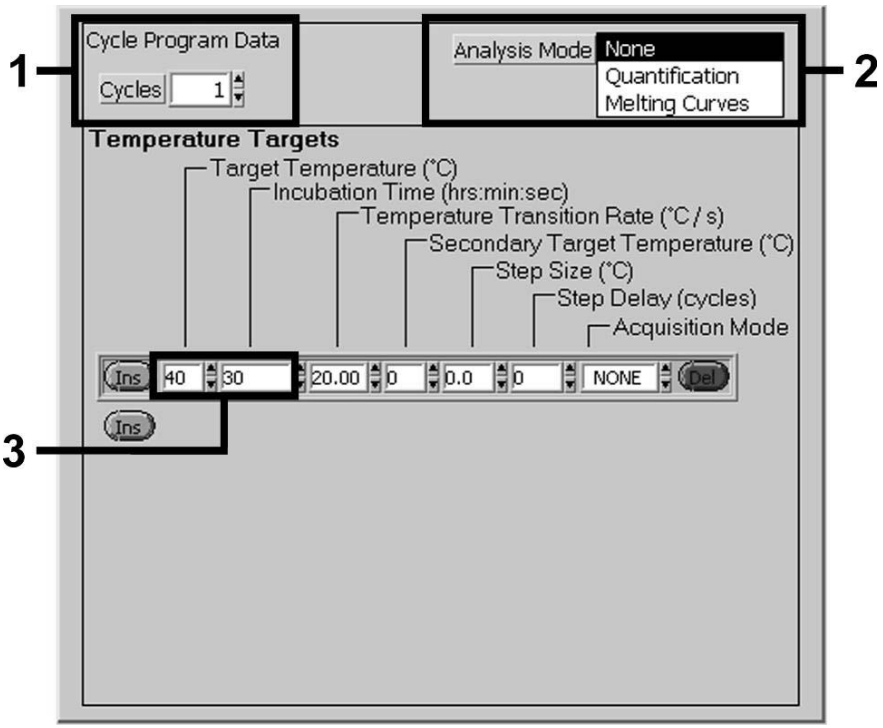

Fig. 11: Cooling.

9. Data Analysis

9.1 Data Analysis for Influenza

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* Influenza/H5 LC RT-PCR Kit please select fluorescence display options F1 for the analytical Influenza RT-PCR and F3/Back-F1 for the *Internal Control* RT-PCR, respectively.

The following results are possible:

1. A signal is detected in fluorimeter channel F1.

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

   In this case, the detection of a signal in the F3/Back-F1 channel is dispensable, since high initial concentrations of influenza virus 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 no influenza virus RNA is detectable. It can be considered negative.

   In the case of a negative Influenza 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**.

An example of positive and negative PCR reactions is given in Fig. 12 and Fig. 13.

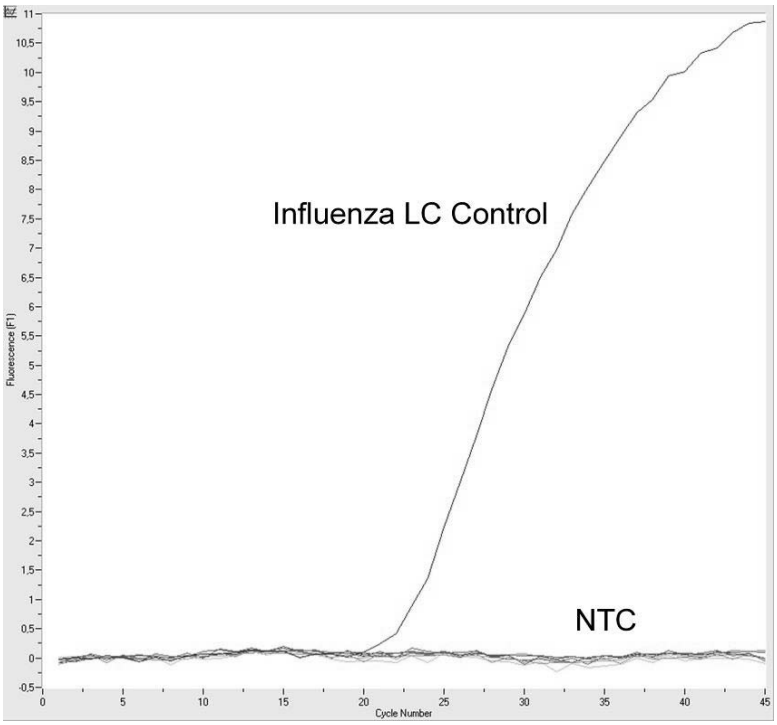

Fig. 12: Detection of the positive controls (*Influenza LC Control*) in fluorimeter channel F1. NTC: non-template control (negative control).

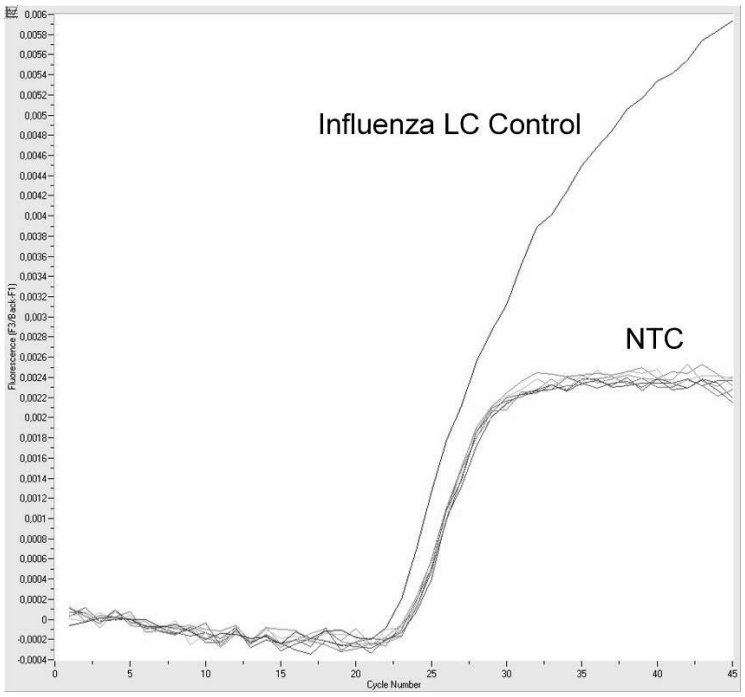

Fig. 13: Detection of the *Internal Control* (*IC*) in fluorimeter channel F3/Back-F1 with simultaneous amplification of the positive control (*Influenza LC Control*). NTC: non-template control (negative control).

9.2 Data Analysis for Influenza/H5

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* Influenza/H5 LC RT-PCR Kit please select fluorescence display option F2/Back-F1 for the analytical Influenza/H5 RT-PCR.

The following results are possible:

1. A signal is detected in fluorimeter channel F2/Back-F1.

   The result of the analysis is positive: The sample contains influenza/H5 virus RNA.

2. In fluorimeter channel F2/Back-F1 no signal is detected.

   In the sample no influenza/H5 virus RNA is detectable. It can be considered negative for influenza/H5. Please note that an influenza/H5 negative sample may still be positive for a non-H5 virus strain.

   If the Influenza/H5 RT-PCR is negative, the detection signal of the Internal Control of the previously run Influenza RT-PCR rules out a possible RT-PCR inhibition.

   Information regarding error sources and their solution can be found in **10. Troubleshooting**.

Examples of positive and negative PCR reactions are given in Fig. 14.

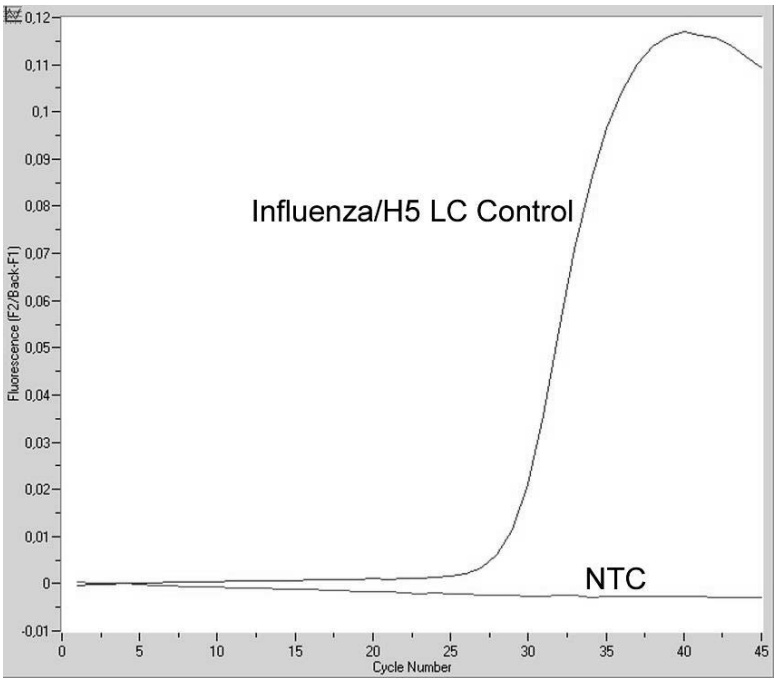

Fig. 14: Detection of the positive control (*Influenza/H5 LC Control*) in fluorimeter channel F2/Back-F1. NTC: non-template control (negative control).

10. Troubleshooting

10.1 Troubleshooting for Influenza RT-PCR

No signal with positive control (*Influenza LC Control*) 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 Influenza 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 **8.4.1 Programming of the Influenza PCR**).
- Incorrect configuration of the PCR reaction.
  - → Check your work steps by means of the pipetting scheme (see **8.3 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* Influenza/H5 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* Influenza/H5 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.

10.2 Troubleshooting for Influenza/H5 RT-PCR

No signal with positive control (*Influenza/H5 LC Control*) in fluorimeter channel F2/Back-F1:

- The selected fluorimeter channel for PCR data analysis does not comply with the protocol.
  - → For data analysis select the fluorimeter channel F2/Back-F1 for the analytical Influenza/H5 RT-PCR.
- Incorrect programming of the temperature profile of the *LightCycler*® Instrument.
  - → Compare the temperature profile with the protocol (see **8.4.2 Programming of the Influenza/H5 PCR**).
- Incorrect configuration of the PCR reaction.
  - → Check your work steps by means of the pipetting scheme (see **8.3 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* Influenza/H5 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 F2/Back-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

The analytical sensitivity of the *artus* Influenza/H5 LC RT-PCR Kit is currently determined in a validation study.

11.2 Specificity

The specificity of the *artus* Influenza/H5 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 subtypes and genotypes has thus been ensured.

11.3 Subtypes

The influenza RT-PCR is designed to detect all influenza A subtypes (H1 - H15 and N1 - N9). Furthermore, all influenza B variants are identified. The influenza/H5 RT-PCR is designed to detect specifically all influenza A subtypes (H5 and N1 - N9). Should you observe any problems in the context of variant detection, please contact our Technical Service.

12. Product Use Limitations

- The *artus* Influenza/H5 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).
- 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* Influenza/H5 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* Influenza/H5 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

Components

Contains

Number

<N>

Contains sufficient for <N> tests

Temperature limitation

Refer to information given in the handbook

IC

Internal Control

Mg-Sol

Magnesium Solution

www.qiagen.com

Australia ■ Orders 03-9840-9800 ■ Fax 03-9840-9888 ■ Technical 1-800-243-066

Austria ■ Orders 0800/28-10-10 ■ Fax 0800/28-10-19 ■ Technical 0800/28-10-11

Belgium ■ Orders 0800-79612 ■ Fax 0800-79611 ■ Technical 0800-79556

Brazil ■ Orders 0800-557779 ■ Fax 55-11-5079-4001 ■ Technical 0800-557779

Canada ■ Orders 800-572-9613 ■ Fax 800-713-5951 ■ Technical 800-DNA-PREP (800-362-7737)

China ■ Orders 021-3865-3865 ■ Fax 021-3865-3965 ■ Technical 800-988-0325

Denmark ■ Orders 80-885945 ■ Fax 80-885944 ■ Technical 80-885942

Finland ■ Orders 0800-914416 ■ Fax 0800-914415 ■ Technical 0800-914413

France ■ Orders 01-60-920-926 ■ Fax 01-60-920-925 ■ Technical 01-60-920-930 ■ Offers 01-60-920-928

Germany ■ Orders 02103-29-12000 ■ Fax 02103-29-22000 ■ Technical 02103-29-12400

Hong Kong ■ Orders 800 933 965 ■ Fax 800 930 439 ■ Technical 800 930 425

Ireland ■ Orders 1800-555-049 ■ Fax 1800-555-048 ■ Technical 1800-555-061

Italy ■ Orders 02-33430411 ■ Fax 02-33430426 ■ Technical 800-787980

Japan ■ Telephone 03-5547-0811 ■ Fax 03-5547-0818 ■ Technical 03-5547-0811

Korea (South) ■ Orders 1544 7145 ■ Fax 1544 7146 ■ Technical 1544 7145

Luxembourg ■ Orders 8002-2076 ■ Fax 8002-2073 ■ Technical 8002-2067

Mexico ■ Orders 01-800-7742-639 ■ Fax 01-800-1122-330 ■ Technical 01-800-7742-639

The Netherlands ■ Orders 0800-0229592 ■ Fax 0800-0229593 ■ Technical 0800-0229602

Norway ■ Orders 800-18859 ■ Fax 800-18817 ■ Technical 800-18712

Singapore ■ Orders 65-67775366 ■ Fax 65-67785177 ■ Technical 65-67775366

Spain ■ Orders 91-630-7050 ■ Fax 91-630-5145 ■ Technical 91-630-7050

Sweden ■ Orders 020-790282 ■ Fax 020-790582 ■ Technical 020-798328

Switzerland ■ Orders 055-254-22-11 ■ Fax 055-254-22-13 ■ Technical 055-254-22-12

UK ■ Orders 01293-422-911 ■ Fax 01293-422-922 ■ Technical 01293-422-999

USA ■ Orders 800-426-8157 ■ Fax 800-718-2056 ■ Technical 800-DNA-PREP (800-362-7737)

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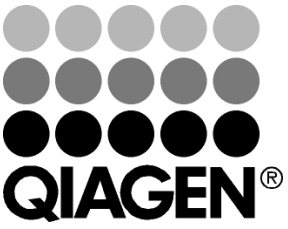

Sample & Assay Technologies