artus® Orthopox LC PCR Kit

Handbook

Σ 24 (catalog no. 4512003)

Σ 96 (catalog no. 4512005)

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 Orthopox LC PCR Kit

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The *artus* Orthopox LC 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.

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1. Contents

	Labelling and contents	Art. No. 4512003 24 reactions	Art. No. 4512005 96 reactions
Blue	*Orthopox LC Master*	2 x 12 rxns	8 x 12 rxns
Yellow	*Orthopox LC Mg-Sol*¤	1 x 400 µl	1 x 400 µl
Red	*Orthopox LC QS 1*¤ *1 x 10*^{4} *cop/µl*	1 x 200 µl	1 x 200 µl
Red	*Orthopox LC QS 2*¤ *1 x 10*^{3} *cop/µl*	1 x 200 µl	1 x 200 µl
Red	*Orthopox LC QS 3*¤ *1 x 10*^{2} *cop/µl*	1 x 200 µl	1 x 200 µl
Red	*Orthopox LC QS 4*¤ *1 x 10*^{1} *cop/µl*	1 x 200 µl	1 x 200 µl
Violet	*Orthopox LC HA for Primer*	1 x 50 µl	1 x 50 µl
Violet	*Orthopox LC HA rev Primer*	1 x 50 µl	1 x 50 µl
Green	*Orthopox 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*
Mg-Sol = *Magnesium Solution*

2. Storage

The components of the *artus* Orthopox LC 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
- DNA isolation kit (see **8.1 DNA Isolation**)
- Pipettes (adjustable)
- Sterile pipette tips with filters
- Vortex mixer
- Desktop centrifuge with rotor for 2 ml reaction tubes
- *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

The *Poxviridae* comprise a family of complex DNA viruses that replicate in the cytoplasm of vertebrate or invertebrate cells. The subfamily *Chordopoxvirinae* (poxviruses of vertebrates) contains eight genera; the medically most important is the genus *Orthopoxvirus*. The genus comprises - among others - four viruses which can infect humans: smallpox virus, cowpox virus, vaccinia virus (the agent used for immunization against smallpox), and monkeypox virus. Smallpox is acquired by inhalation of air droplets or aerosols. Twelve to fourteen days after infection, the patient typically becomes febrile and has severe aching pains and prostration. Some two to three days later, a papular rash develops over the face and spreads to the extremities. The rash soon becomes vesicular and later, pustular. The patient remains febrile throughout the development of the rash and customarily experiences considerable pain as the pustules grow and expand. Gradually, scabs form, which eventually separate, leaving pitted scars. Smallpox is fatal in 40 % of all patients who die in the second week after onset of symptoms.

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* Orthopox LC PCR Kit constitutes a ready-to-use system for the detection of orthopoxvirus DNA using polymerase chain reaction (PCR) and following *melting curve* analysis in the *LightCycler*® Instrument. The *Orthopox LC Master* contains reagents and enzymes for the specific amplification of a 110 bp region of the Orthopox genome, and for the direct detection of the specific amplicon in fluorimeter channel F2/Back-F1 of the *LightCycler*® Instrument. In addition, the *artus* Orthopox LC 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 Orthopox PCR is not reduced. In order to distinguish smallpox virus from all other species of the genus, the system utilizes the specific melting temperatures of the probes. During the curve analysis, a signal is detected in fluorimeter channel F2/Back-F1 for the genus *Orthopoxvirus* at 65°C and for variola major virus (also variola minor virus) at 55°C. Depending on the various extraction conditions and as a result of their respective buffering conditions, these values can deviate by 1 - 2°C. External positive controls (*Orthopox LC QS 1 - 4*) are supplied which allow the determination of the amount of viral DNA. For further information, please refer to section **8.3 Quantitation**. Please note that the reagents are supplied only with vaccinia virus derived positive controls. No variola specific positive control material is included.

Furthermore the system includes a second "conventional" amplification system (primer set) located in the hemagglutinin (HA) gene of the virus for a further proof of virus identity by conventional methods. Please contact our Technical Service for additional information.

Attention: It is emphasized that under **any** circumstances a positive PCR must be confirmed by another method. Depending on the sample material, sequencing, electron microscopy analysis or antigen detection methods should be used for confirmation. At least two additional gene segments should be sequenced for approval of the results.

8. Protocol

8.1 DNA Isolation

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

Sample Material	Nucleic Acid Isolation Kit	Catalogue Number	Manufacturer	Carrier RNA
Plasma, serum	QIAamp UltraSens Virus Kit (50)	53 704	QIAGEN	included
Blood, scabs	QIAamp DNA Mini Kit (50)	51 304	QIAGEN	not included

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

Number of samples	1	12
Lysis buffer	e.g. 200 µl	e.g. 2,400 µl
Carrier RNA (1 µg/µl)	2 µl	24 µl
Total Volume	**202 µl**	**2,424 µl**
Volume per extraction	**200 µl**	**each 200 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 use of **carrier RNA** is critical for the extraction efficiency and, consequently, for DNA/RNA yield. To increase the stability of the carrier RNA provided with the QIAamp UltraSens Virus Kit, we recommend the following procedure deviant from the user manual of the extraction kit:

  a. Resuspend the lyophilised carrier RNA prior to first use of the extraction kit in 310 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 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**

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

- It is recommended to elute the DNA in 50 µl elution buffer to get the highest sensitivity of the *artus* Orthopox LC PCR Kit.

- The **QIAamp UltraSens Virus Kit** allows a sample concentration. If you use sample material other than serum or plasma, please add 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* Orthopox LC PCR Kit should not be used with **phenol**-based isolation methods.

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

8.2 Internal Control

An *Internal Control* (*Orthopox LC IC*) is supplied. This allows the user **both to control the DNA isolation procedure and to check for possible PCR inhibition** (see Fig. 1). For this application, add the *Internal Control* to the isolation at a ratio of 0.1 µl per 1 µl elution volume. For example, using the QIAamp UltraSens Virus Test Kit, the DNA is eluted in 50 µl AVE buffer. Hence, 5 µl of the *Internal Control* should be added initially. The quantity of *Internal Control* used depends **only** on the elution volume. The *Internal Control* and carrier RNA (see **8.1 DNA Isolation**) should be added only

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

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

The *Internal Control* can optionally be used **exclusively to check for possible PCR inhibition** (see Fig. 2). For this application, add 0.5 µl of the *Internal Control* and 2 µl *Orthopox LC Mg-Sol* per reaction directly to 13 µl *Orthopox 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 *Orthopox LC Master*, *Orthopox LC Mg-Sol* and the *Internal Control* according to the number of samples (see **8.4 Preparing the PCR**).

8.3 Quantitation

The enclosed *Quantitation Standards* (*Orthopox 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* of *Orthopoxvirus* (*Orthopox LC QS 1 - 4*) 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:

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

* 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.

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

Important: A guideline for the quantitative analysis of *artus* systems on the *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* (*Orthopox LC QS 1 - 4*) as well as one negative control (*Water*, *PCR grade*) are included per PCR run. To generate a standard curve, use all supplied *Quantitation Standards* (*Orthopox 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 quick vortexing) and centrifuged briefly.

If you want to use the *Internal Control* **to monitor the DNA isolation procedure and to check for possible PCR inhibition,** it has already been added to the isolation (see **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	*Orthopox LC Master*	13 µl	156 µl
	Orthopox LC Mg-Sol	2 µl	24 µl
	Orthopox 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 *Orthopox 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	*Orthopox LC Master*	13 µl	156 µl
	Orthopox LC Mg-Sol	2 µl	24 µl
	Orthopox 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 DNA. Correspondingly, 5 µl of at least one of the *Quantitation Standards* (*Orthopox 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

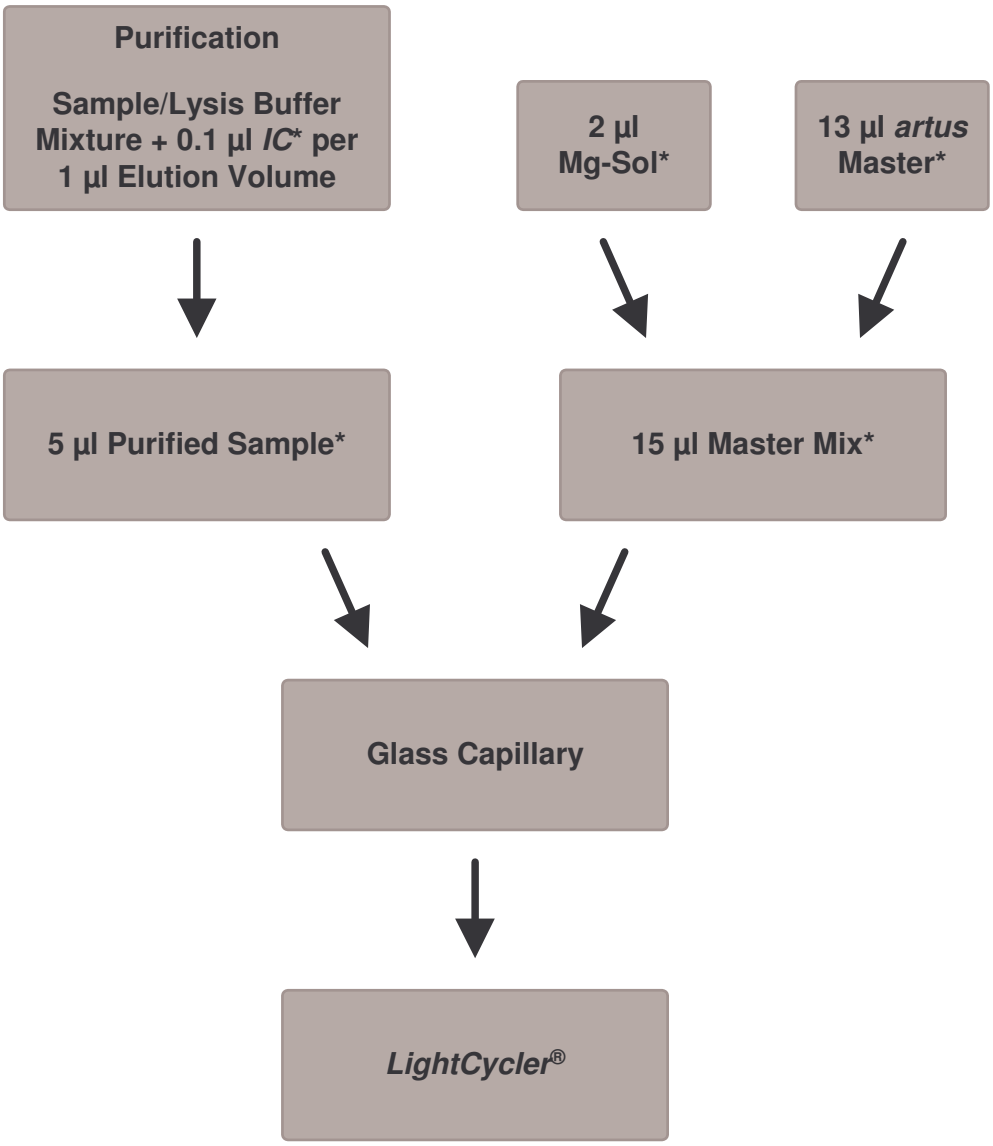

Fig. 1: Schematic workflow for the control of both the purification procedure and PCR inhibition.

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

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

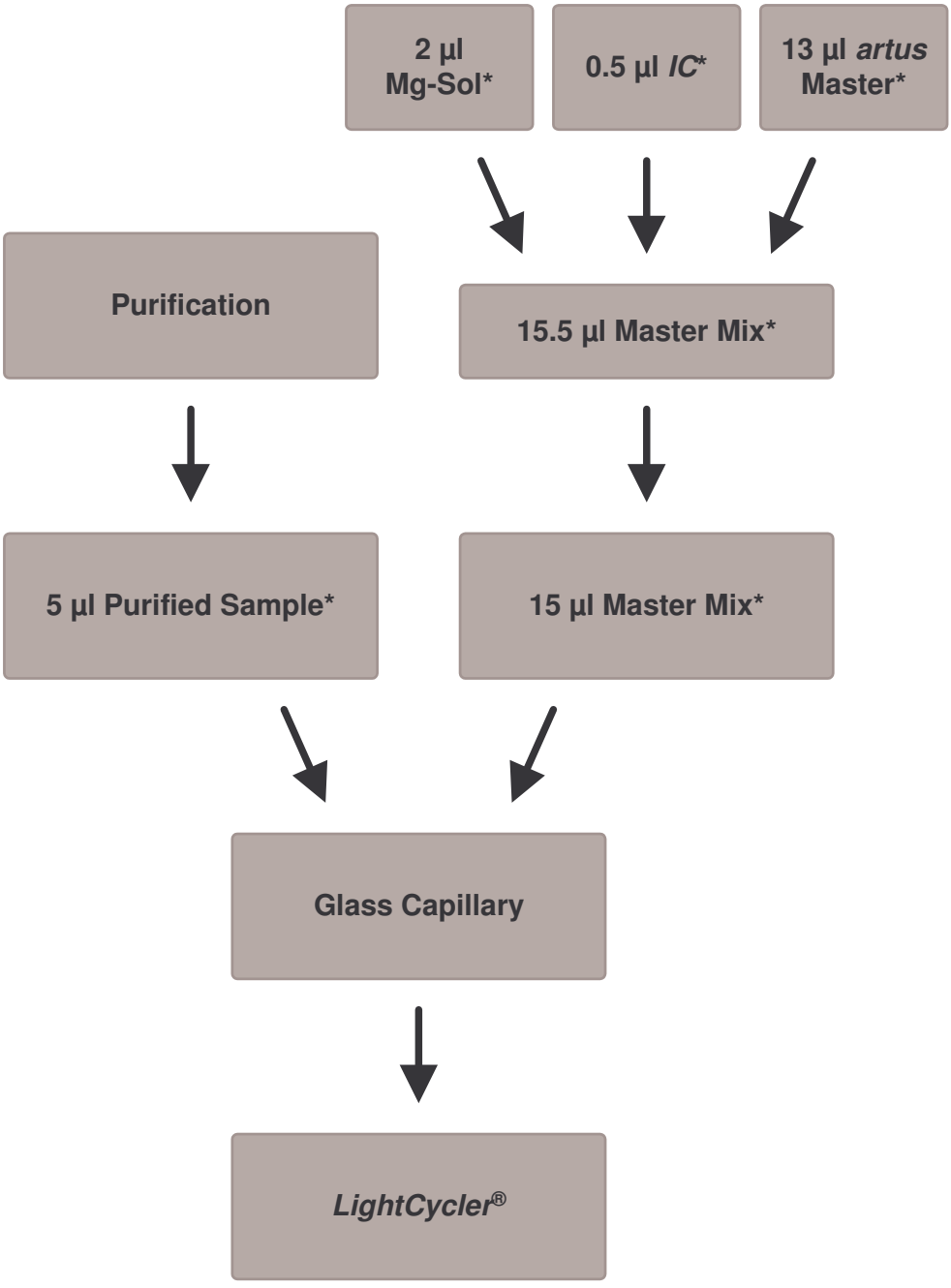

Fig. 2: Schematic workflow for the control of PCR inhibition.

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

8.5 Programming the *LightCycler*® Instrument

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

A.	Initial Activation of the Hot Start Enzyme	Fig. 3
B.	Amplification of the DNA	Fig. 4
C.	Melting Curve	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*.

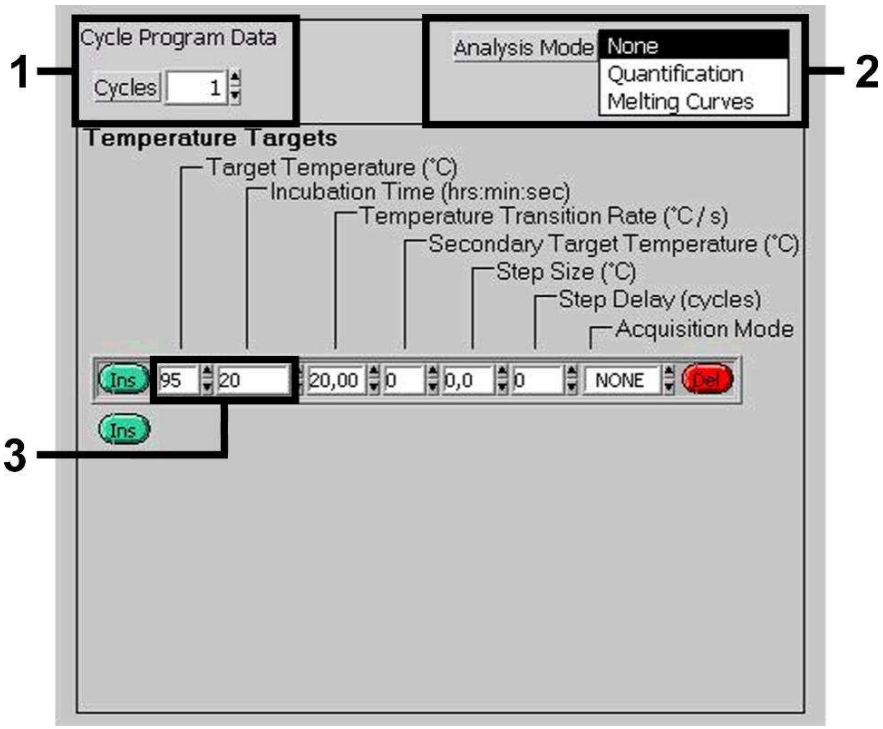

Fig. 3: Initial Activation of the Hot Start Enzyme.

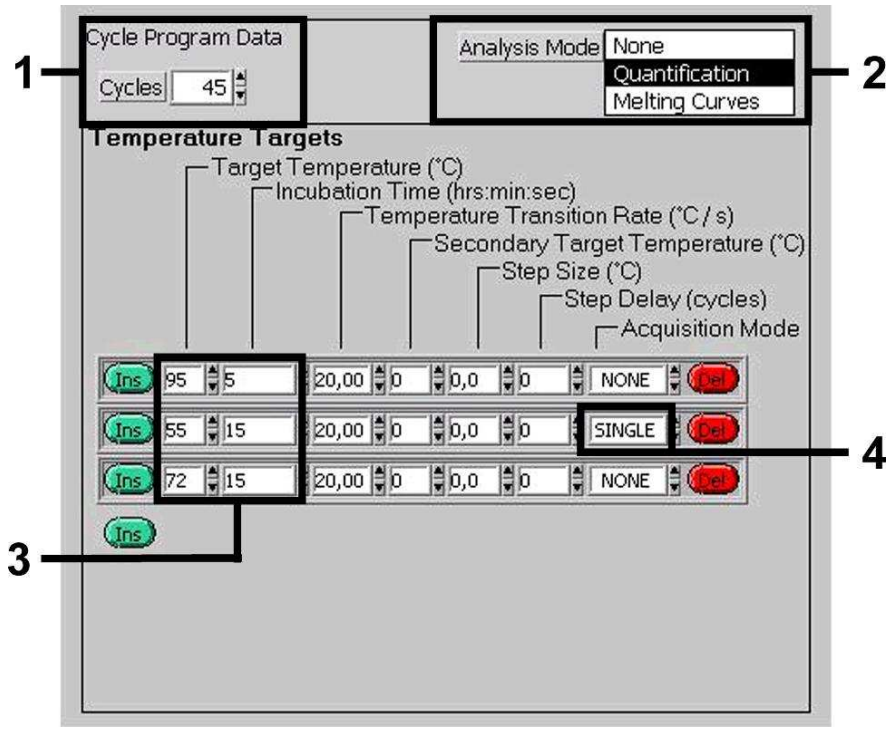

Fig. 4: Amplification of the DNA.

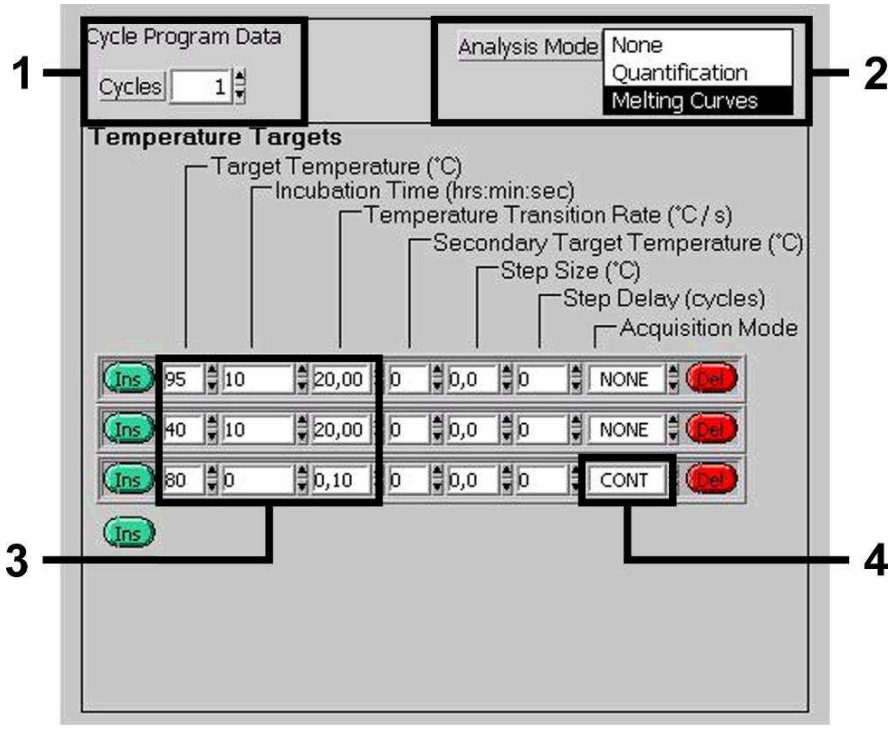

Fig. 5: Melting Curve.

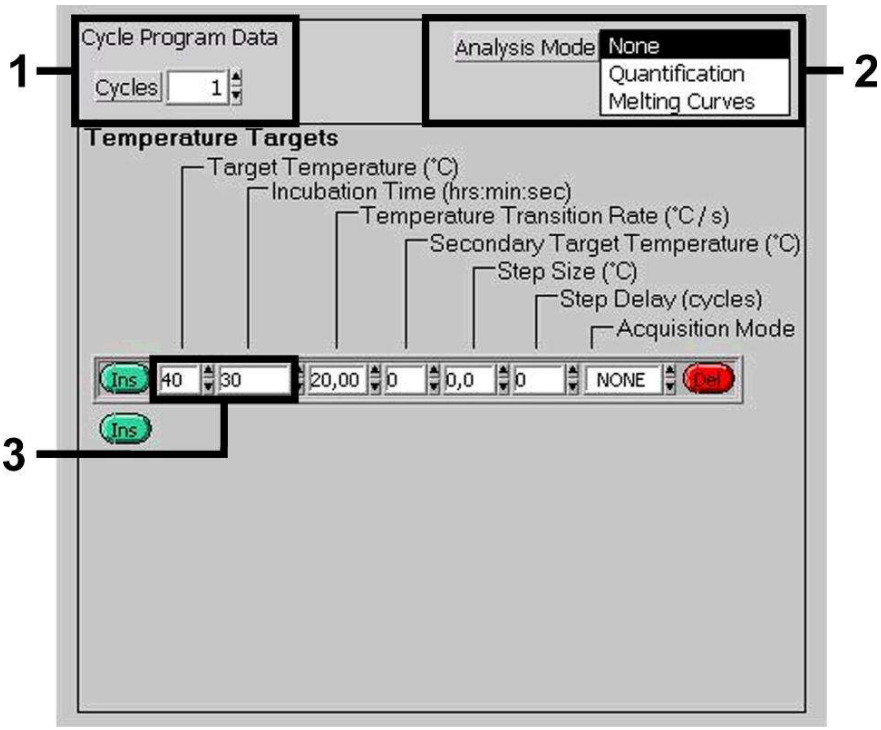

Fig. 6: Cooling.

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* Orthopox LC PCR Kit please select fluorescence display options F2/Back-F1 for the analytical Orthopox PCR and F3/Back-F1 for the *Internal Control* 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 F2/Back-F1.

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

   In this case, the detection of a signal in the F3/Back-F1 channel is dispensable, since high initial concentrations of orthopoxvirus DNA (positive signal in the F2/Back-F1 channel) can lead to a reduced or absent fluorescence signal of the *Internal Control* in the F3/Back-F1 channel (competition).

Attention: It is emphasized that under **any** circumstances a positive PCR must be confirmed by another method. Depending on the sample material, sequencing, electron microscopy analysis or antigen detection methods should be used for confirmation. At least two additional gene segments should be sequenced for approval of the results.

Differentiation can be made between the orthopoxviruses other than variola virus and variola virus amplicons on the basis of a melting curve analysis (channel F2/Back-F1, programme *melting curve*); the melting peak (T_m) for orthopoxviruses should be at 65°C and for variola virus at 55°C. Depending on the extraction conditions and as a result of their respective buffering conditions, these values can deviate by 1 - 2°C. However, this deviation will be equal for both melting peaks.

2. In fluorimeter channel F2/Back-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 orthopoxvirus DNA is detectable. It can be considered negative.

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

3. No signal is detected in the F2/Back-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, 8 and 9.

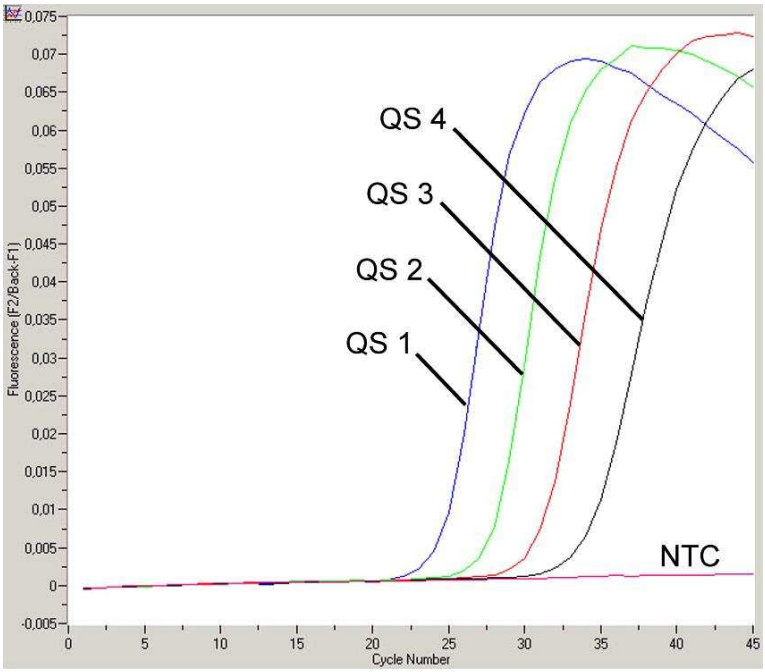

Fig. 7: Detection of the *Quantitation Standards* (*Orthopox LC QS 1 - 4*) in fluorimeter channel F2/Back-F1. NTC: non-template control (negative control).

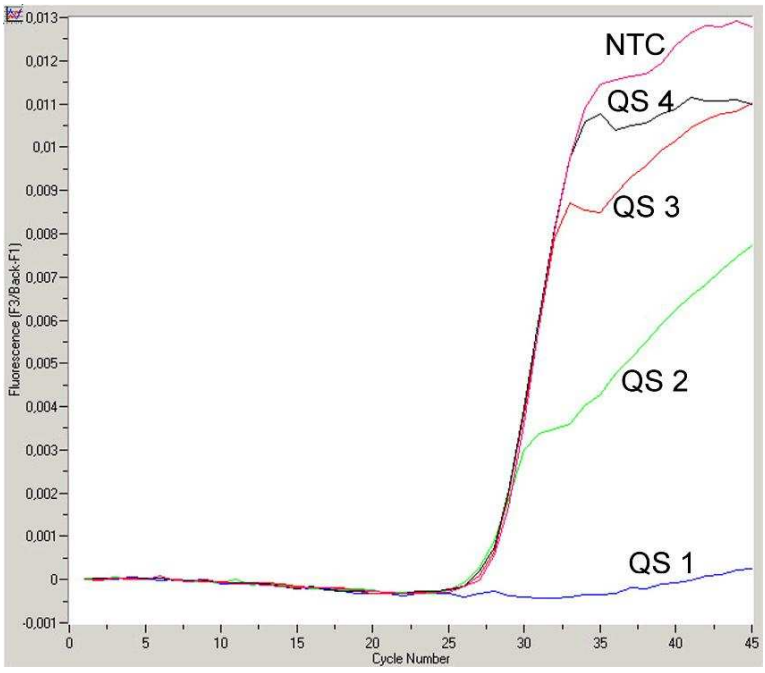

Fig. 8: Detection of the *Internal Control* (*IC*) in fluorimeter channel F3/Back-F1 with simultaneous amplification of *Quantitation Standards* (*Orthopox LC QS 1 - 4*). NTC: non-template control (negative control).

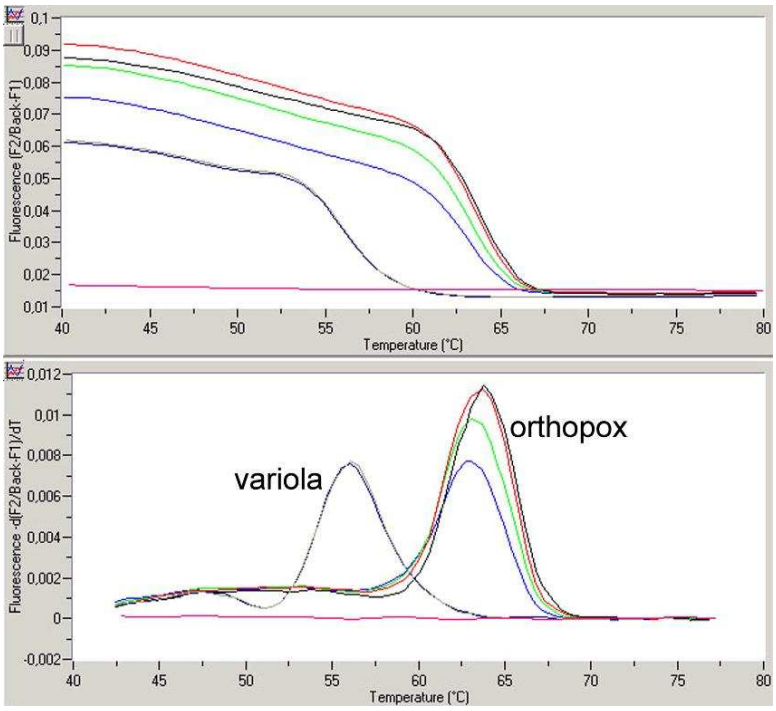

Fig. 9: Differentiation between the group of orthopoxviruses and the variola species in fluorimeter channel F2/Back-F1 (programme *Melting Curve*).

10. Troubleshooting

No signal with positive controls (*Orthopox LC QS 1 - 4*) 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 Orthopox PCR and the fluorimeter channel F3/Back-F1 for the *Internal Control* 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* Orthopox LC 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 F2/Back-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 DNA Isolation**) and stick closely to the manufacturer's instructions.

→ Make sure that during the DNA isolation the recommended additional centrifugation step has been carried out before the elution in order to remove any residual ethanol (see **8.1 DNA Isolation**).

- DNA was lost during extraction.
  → If the *Internal Control* had been added to the extraction, an absent signal of the *Internal Control* can indicate the loss of DNA during the extraction. Make sure that you use a recommended isolation method (see **8.1 DNA Isolation**) and stick closely to the manufacturer's instructions.
- The storage conditions for one or more kit components did not comply with the instructions given in **2. Storage** or the *artus* Orthopox LC 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 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

The reagents have been validated in collaboration with leading experts in the field of *Orthopoxvirus* research. The results are published. It is possible to request a reprint at **info-hamburg@qiagen.com**.

12. Product Use Limitations

- The *artus* Orthopox LC 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* Orthopox LC 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* Orthopox LC PCR Kit is tested against predetermined specifications to ensure consistent product quality.

15. References

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

(2) Niedrig M, Schmitz H, Becker S, Günther S, Meulen J, Meyer H, Ellerbrock H, Nitsche A, Gelderblom HR, Drosten Ch. First International Quality Assurance Study on the Rapid Detection of Viral Agents of Bioterrorism. J Clin Microbiol 2004; 42 (4): 1753 - 1755.

(3) Olson VA, Laue T, Laker MT, Babkin I, Drosten Ch, Shchelkunov SN, Niedrig M, Damon IK, Meyer H. Real-Time PCR System for Detection of Orthopoxviruses and Simultaneous Identification of Smallpox Virus. J Clin Microbiol 2004; 42 (5): 1940 - 1946.

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

Mg-Sol

Magnesium Solution

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Sample & Assay Technologies