ANCHOR

Monkey-/Orthopoxvirus PCR Kit

Instructions for Use

Anchor Monkey-/Orthopoxvirus PCR Kit

RUO

Qualitative Real-Time PCR Kit

for Research Use Only

RUO For Research Use Only	QG A2611-UK - 05.05.2023
REF A2600	-30°C to -15°C
Σ 100	ANCHOR Diagnostics GmbH Grandweg 64 D-22529 Hamburg
HB A2610-UK - 18.08.2024	

compatible with

LightCycler 480 II (Roche)

cobas z 480 Analyzer (Roche)

CFX96 (Bio-Rad)

Rotor-Gene Q (QIAGEN)

QuantStudio 5 (Applied Biosystems)

Mic qPCR (Biomolecular Systems)

1 Contents

2 Product Description

The Anchor Monkey- / Orthopoxvirus PCR Kit is a Real-Time PCR technology-based test for the amplification, detection and differentiation of nucleic acids (double-stranded RNA binding protein) from monkeypox virus and other members of the *Orthopoxvirus* genus.
In addition, a heterologous amplification system (Internal Control) is included to supervise the success of the sample extraction procedure and to identify possible inhibition of the amplification reaction. Probes linked to distinguishable fluorescent dyes enable the parallel detection and differentiation of monkeypox virus and *Orthopoxvirus*-specific nucleic acids and the Internal Control in three corresponding detector channels of the Real Time PCR instrument. The *Orthopoxvirus*-specific PCR system does not detect monkeypox virus DNA which is detected in the corresponding detection channel. Modified-Vaccinia-Ankara Virus (MVA) which is the vaccine strain of the IMVANEX Smallpox vaccine, would be detected as *Orthopoxvirus*.
The Positive Control contains artificial DNA bearing the monkeypox virus and orthopoxvirus target sequences. They can be used together with the Negative Control DNA 2 to monitor the integrity of the analyte-specific reagents of the kit and the proper performance of the reaction.

3 Kit Components

Master A and Master B reagents contain all necessary components (PCR buffer, Polymerase, magnesium ions, dNTPs, primers, and probes) to allow PCR mediated amplification and target detection of monkeypox virus and *Orthopoxvirus* specific DNA and Internal Control in one reaction setup.
The PC (Positive Control) and NC (Negative Control) DNA 2 are supplied with the IC (Internal Control) DNA 2 already incorporated (see also section 8.2.1 Master Mix Set-Up).

The reagents provided with the kit allow the preparation of 100 reactions.

Master A Monkey-/ Orthopox	Master B Monkey-/ Orthopox	IC DNA 2	! PC Monkey-/ Orthopox	! NC DNA 2
A2601	A2602	A0022	A2603	A0032
4 Vials	4 Vials	1 Vial	1 Vial each	1 Vial
4x 125 µL	4x 125µL	1000 µL	1x 200 µL	200 µL
Contains: Buffer, Bovine Serum Albumin, Polymerase	Contains: Buffer, Salt, Nucleotides, Target- and IC-specific Oligonucleotides	Contains: Buffer, IC-specific synthetic Polynucleotide	Contains: Buffer substance, Target-specific synthetic Polynucleotide	Contains: Buffer substance, IC-specific synthetic Polynucleotide

! INTERNAL CONTROL INSIDE !

4 Storage and Stability

- The Anchor Monkey-/Orthopoxvirus PCR Kit is shipped on dry ice and should be stored at –30 to –15°C upon receipt.
- Store monkeypox virus and/or *Orthopoxvirus* DNA-positive and/or potentially positive materials separated from the kit.
- Repeated thawing and freezing of the Master reagents of > 3x should be avoided, as this may reduce the assay performance.
- For the PC, the NC DNA 2 and the IC DNA 2, thawing and freezing cycles up to 4x are allowed. Alternatively, storage between +2 to +8°C for up to 14 days is possible.
- Due to the components used it might be possible that Master vials do not always freeze completely after initial thawing. This is not a matter of concern and does not influence the stability or performance of the assay.
- If the reagents are to be used only intermittently, they should be frozen in aliquots. Label aliquots clear and unambiguously to avoid a mix-up of reagents.
- During PCR set up the reagents should be kept cooled at +2 to +8°C – use cooling block.
- Do not store Master A and Master B Monkey-/Orthopox more than 3 h at +2 to +8°C.
- Protect all reagents from extensive light exposure.

5 Material Required but Not Provided

- Nucleic acid purification system
- Real Time PCR instrument
- Appropriate PCR reaction vessels and related accessories
- Cooling block (for reaction setup)
- Benchtop centrifuge (rotor holding 2 mL reaction tubes)
- Vortex mixer
- Pipettes (variable volume)
- Single-use pipette filter tips
- 1.5 mL or 2 mL reaction tubes (for Master mix set-up)
- Single-use gloves (powder-free)

Use all materials and equipment according to the manufacturer's instructions. Maintain and calibrate the equipment as recommended by the manufacturer.

6 Limitations

- Strict compliance with the Instructions for Use is required for optimal PCR results.
- The presence of PCR inhibitors may cause invalid results.
- Occurrence of mutations within the target region might result into a reduced sensitivity or a complete detection failure.
- Following good laboratory practices is crucial for the successful usage of the product.
- Appropriate handling of the reagents is essential to avoid contaminations or impurities.

7 Warnings and Precautions

- For research use only. Not for use in diagnostic procedures.
- Use of this product is recommended to personnel specially instructed and trained in the techniques of Real Time PCR.
- Specimens should always be treated as potentially infectious and/or biohazardous material in accordance with safe laboratory procedures.
- Wear protective single-use gloves, a laboratory coat and eye protection when handling specimens or kit components.
- Avoid microbial and nuclease (DNase/RNase) contamination of the specimen and the components of the kit.
- Always use DNase/RNase-free single-use pipette tips with aerosol barriers.
- Use separated working areas for (1) specimen preparation, (2) PCR reaction set-up and (3) amplification/detection activities.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Do not open the reaction tubes/plates post amplification, to avoid contamination with amplicons.
- Discard sample and assay waste according to your local safety regulations.

8 Workflow

8.1 Sample Preparation

8.1.1 Sample Preparation

Purified DNA is the sample input material for the Anchor Monkey-/Orthopoxvirus PCR Kit. It has to be ensured that the chosen nucleic acid purification method is compatible with Real-Time PCR technology. The extraction has to be executed according to the manufacturer's instructions.

ⓘ If sample eluates are not directly used for PCR analysis, store eluates at -30 to -15 °C. In case of using eluates repeatedly, avoid frequent thaw/freeze cycles (not more than 3 cycles).

ⓘ Eluates should be labelled clearly and unambiguously to avoid a mix-up of samples.

8.1.2 Internal Control

The Internal Control DNA 2 provided with the Anchor Monkey-/Orthopoxvirus PCR Kit should be co-purified with the nucleic acid of interest to monitor sample preparation efficiency and quality.

ⓘ The Internal Control DNA 2 MUST NOT be added directly to the sample.

Always add the Internal Control DNA 2 after lysis buffer has been added to the sample.
The required volume of Internal Control DNA 2 per sample purification is defined by the chosen elution buffer volume.

Ten percent of the elution buffer volume used should be added to the sample/lysis mixture.

Examples:

- Elution buffer per sample: 200 µL -> IC DNA 2 volume: 20 µL
- Elution buffer per sample: 60 µL -> IC DNA 2 volume: 6 µL

ⓘ Secure the elimination of residual ethanol before elution of nucleic acids. Ethanol may inhibit the amplification process.

If no co-purification of the Internal Control is planned and the IC DNA 2 should be used only as an inhibition control of the reaction, either the amount of IC related to the used elution volume could be added to each eluate or 1.5 µL of the IC DNA 2 / per reaction should be added to the master mix (see section 8.2.1 Master Mix Set-Up).

8.2 PCR Preparation

8.2.1 Master Mix Set-Up

ⓘ Consider configuring the run settings of the PCR cycler software to have the instrument ready before starting the PCR reaction preparation (refer to section 8.3 PCR Cycler Configuration).

Prepare the Master Mix step by step:

- Thoroughly thaw Master A and B.
- Mix Master A and B by gentle pipetting or short pulse-vortexing.
- Spin Master A and B shortly with a benchtop centrifuge to remove residual droplets from tube lids.
- According to your preferred workflow follow one of the pipette schemes below to mix Master A and B using a 1.5 mL or 2 mL reaction tube:

IC DNA 2 present in sample eluates – NO IC DNA 2 added to Master Mix preparation:

Number of reactions	1	10(+1)*	N**
Master A Monkey-/Orthopox	5 µL (X)	55 µL	Y µL
Master B Monkey-/Orthopox	5 µL (X)	55 µL	Y µL
Volume Master Mix	10 µL	110 µL	Z µL

*10 reactions + 10%
** See formula on next page

IC DNA 2 to be used as inhibition control only – IC DNA 2 added to Master Mix preparation:

Number of reactions	1	10(+1)*	N**
Master A Monkey-/Orthopox	5 µL (X)	55 µL	Y µL
Master B Monkey-/Orthopox	5 µL (X)	55 µL	Y µL
IC DNA 2	1.5 µL (X)	16.5 µL	Y µL
Volume Master Mix	11.5 µL	126.5 µL	Z µL

*10 reactions + 10%
** See formula on next page

ⓘ We recommend calculating for an additional volume of at least 10% to compensate potential loss during pipetting. The needed volume will be calculated by using the following formula:

** $N \times X\mu L x 1.1 = Y$

N = Number of reactions
X = Volume of component per reaction
Y = Total volume of component
Z = Total volume of Master Mix

- Mix prepared Master Mix by gentle and short pulse-vortexing.
- Spin Master Mix shortly with a benchtop centrifuge to remove residual droplets from tube lids.

ⓘ It is recommended to test the Positive Control and the Negative Control at least once in each PCR run.

ⓘ The PC and the NC DNA 2 already contain the IC DNA 2 in a ready-to-use concentration. No addition of IC necessary!

If you want to use a Master Mix preparation with added IC DNA 2 (as inhibition control) in combination with the PC and NC DNA 2, be aware that the IC signal of the controls will slightly shift towards a lower CT value in comparison to the IC signal of the controls using a Master mix without additional IC.

8.2.2 PCR Reaction Set-Up

ⓘ Always use a cooling block for the preparation of the PCR reaction mix.

Prepare the Reaction Mix step by step:

- If previously stored frozen, thaw eluates containing nucleic acid (and IC DNA 2) thoroughly.
- Mix eluates by gentle pipetting or brief pulse-vortexing.
- Spin eluates shortly with a benchtop centrifuge to remove residual droplets from tube lids.
- Pipette **10 µL of Master Mix** (see section 8.2.1 Master Mix Set-Up) into suitable reaction vessels for PCR analysis. This is also valid for Master Mix spiked with IC DNA 2.
- Add **15 µL of eluate** or control (PC Monkey-/Orthopox or Negative Control DNA 2). **Mix well by repeated up and down pipetting!**
- Close reaction vessels securely with the appropriate sealing system.
- Immediately transfer closed and ready-to-use reaction vessels to the Real Time PCR instrument. Avoid any delays!

ⓘ Carefully handle reaction vessels during transfer to avoid mix up of samples.

ⓘ **Complete mixing of Master Mix reagents with a sample or control during reaction set up should be unconditionally secured by repeated up and down pipetting!**

This is essential to achieve an optimum amplification curve performance !!!

Master Mix	+	Eluate / Control	=	Reaction Mix
10 µL		15 µL		25 µL

8.3 PCR Cycler Configuration

The Anchor Monkey-/Orthopoxvirus PCR Kit has been evaluated in combination with the following different PCR Cycler platforms:

PCR Cycler Platform	Run Time
QuantStudio 5 (Applied Biosystems)	≈ 28 min.
LightCycler 480 II (Roche)	≈ 30 min.
Cobas z 480 Analyzer (Roche)	≈ 30 min.
CFX96 (Bio-Rad)	≈ 33 min.
Rotor-Gene Q (QIAGEN)	≈ 44 min.
Mic qPCR (BMS)	≈ 34 min.

The listed run times for the different instruments are effectively measured durations and can differ from what is displayed on the graphical user interface of the individual instrument software. For basic information concerning set-up and programming of the respective Real Time PCR instrument, refer to the instrument-specific manual.

8.3.1 General PCR Cycler Settings

Temperature cycling profile for **QuantStudio 5, LightCycler 480 II, Cobas z 480 Analyzer, CFX96 and Rotor-Gene Q:**

Cycling	95°C	1 sec	x 40
	65°C *	2 sec	
	72°C	1 sec	

* Fluorescence acquisition for Monkeypox-, *Orthopoxvirus* and IC

Temperature cycling profile for **Mic qPCR:**

Cycling	95°C	1 sec	x 40
	63°C *	2 sec	
	72°C	1 sec	

* Fluorescence acquisition for monkeypox virus, *Orthopoxvirus* and IC

Reaction Volume: 25 µL

8.3.2 Specific PCR Cycler Settings

The following table contains PCR cycler-specific recommendations for the basic configuration of the run settings.
For additional information regarding the cycler settings, recommended plastics, colour compensation, gain optimisation settings, etc. do not hesitate to contact us directly (see section 9 Technical Assistance & Contact Information).

Instrument	Target	Detection channel	Recommendations / Requirements
LightCycler® 480 II (Cobas z 480 Analyzer)	Monkey-poxvirus	465/510	**Run Settings:** ▪ Block size: 96 ▪ If clear plates are used, the sensor of the LightCycler® has to be disabled by selecting the Clear Plates option in the software before the run is started. **Consumables:** ▪ LC480 Multiwell Plate 96, white Roche Mat. No. 04729692001) ▪ LC480 Multiwell Plate 96, clear (Roche Mat. No. 05102413001) ▪ LC480 Sealing Foil (Roche Mat. No. 04729757001)
	IC	533/580 (540/580)	
	Ortho-poxvirus	618/660 (610/645)	
Bio-Rad CFX96	Monkey-poxvirus	FAM	**Consumables:** ▪ Hard Shell 96-well PCR Plate, white (Mat. No. HSP9655) ▪ Optical flat 8 Cap Strip for 0.2ml (Mat. No. TCS0803) ▪ 0.2 ml 8-Tube PCR Strips without Caps, low profile, white (Bio-Rad Mat. No. TLS 0851) ▪ 8-strip optical clear flat caps (Sarstedt Mat. No.65.1998.400)
	IC	HEX	
	Ortho-poxvirus	TEXAS RED	

Instrument	Target	Detection channel	Recommendations / Requirements
Rotor-Gene Q	Monkey-poxvirus	Green	**Run Settings:** ▪ Use 72-Well Rotor ▪ Perform Auto-Gain optimisation before 1 st acquisition. **Consumables:** ▪ Strip Tubes and Caps, 0.1 ml (QIAGEN Mat. No 981103)
	IC	Yellow	
	Ortho-poxvirus	Orange	
QuantStudio™ 5	Monkey-poxvirus	FAM	**Run Settings:** ▪ Block Type: 96-Well 0.1-mL Block ▪ Experiment Type: Standard Curve Chemistry: TaqMan® Reagents ▪ Run Mode: Fast ▪ Plate attributes: Passive Reference - None **Consumables:** ▪ 96-Well Fast Thermal Cycling Plates (Life Technologies Mat.No. 4346907) ▪ MicroAmp™ Optical Adhesive Film (Life Technologies Mat. No. 4311971)
	IC	HEX	
	Ortho-poxvirus	TEXAS RED	
Mic qPCR	Monkey-poxvirus	Green	**Run Settings:** ▪ Temperature Control: Standard TAQ **Consumables:** ▪ Mic Tubes and Caps (Mat. No.68MIC-60653)
	IC	Yellow	
	Ortho-poxvirus	Orange	

8.4 Data Analysis

The following table contains cycler-specific references for the configuration of analysis settings. They could serve as an initial orientation. Depending on local cycler- and workflow-related differences adaptations might be necessary. For additional information concerning data analysis, refer to the instrument-specific manual of the respective Real Time PCR instrument or contact us (see section 9 Technical Assistance & Contact Information).

Instrument	Recommendations
LightCycler® 480 II (Cobas z 480 Analyzer)	**Analysis Settings:** ▪ Abs Quant/2nd Derivative Max ▪ Color Comp (off) ▪ Mean ▪ High Confidence
Bio-Rad CFX96	**Analysis Settings (all channels):** ▪ Baseline Substracted Curve Fit ▪ C(t) Determination Mode: Single Threshold ▪ Baseline Threshold: - Baseline Cycles: Auto Calculated
Rotor-Gene Q	**Analysis Settings (all channels):** ▪ Quantitation ▪ Linear Scale ▪ Dynamic Tube ON
QuantStudio™ 5	**Analysis Settings (all channels):** ▪ Plot Type: ΔRn vs Cycle ▪ Graph Type: Linear ▪ Baseline Start/End: 3/15
Mic qPCR	**Analysis Settings (all channels):** ▪ Graph Type: Linear ▪ Method: Dynamic ▪ Ignore Cycles Before: 3 ▪ Threshold Start: 1 ▪ Exclusion: None

[1] Cycler- or run file-specific threshold settings might be necessary

8.4.1 Qualitative Analysis

For a valid run and as a prerequisite for the interpretation of the sample results, the following requirements have to be met by the included kit controls:

Channel/Target	Monkeypoxvirus	Orthopoxvirus	IC
PC Monkey-/Orthopox	+	+	+
NC DNA 2	-	-	+

If one of the conditions has failed, result interpretation of the sample results might be flawed. In case of kit control failure, it is recommended to repeat the PCR run.

In case of a valid run, the following result interpretation can be made:

Result	Monkeypox	Orthopox	IC
Monkeypox DNA positive	+	-	+/-
Non-Monkeypox Orthopox-virus DNA positive	-	+	+/-
Monkeypox and Non-Monkeypox Orthopoxvirus DNA positive (mixed infection)	+	+	+/-
Monkey-/Orthopoxvirus DNA negative	-	-	+
Invalid	-	-	-

A positive result for monkeypox virus and/or *Orthopoxvirus* DNA does not necessarily require a positive signal for the IC since high concentrations of the respective target nucleic acid can result in a competitive inhibition of the IC amplification.

An invalid result for a sample can be due to PCR inhibition or a failure during the nucleic acid isolation procedure. In such cases, it is recommended to dilute the nucleic acid extract 1:10 (recommended to be done in elution buffer, if possible) for a PCR retest or to repeat the nucleic acid isolation procedure. Note that the dilution of the nucleic acid extract might also lead to a reduction of the target nucleic acid concentration below the limit of detection of the Anchor Monkey-/Orthopoxvirus PCR Kit.

9 Technical Assistance & Contact Information

For any questions, a need for technical assistance or if you identify difficulties using our products do not hesitate to contact us:
phone: +49 40 520 14 830
email: support@anchor-diagnostics.com

10 Symbols

RUO	For research use only
REF	Product - Catalogue number
Σ	Contains sufficient reagents for <N> tests
H B	Instructions for Use - Catalogue number and version
	Consult Instructions for Use
QG	Quick Guide - Catalogue number and version
	Temperature limits for storage
	Use by
LOT	Batch code
i	Important Note
	Manufacturer

ANCHOR
Diagnostics GmbH

Grandweg 64
22529 Hamburg | Germany
phone: +49 40 520 148 30
fax: +49 40 520 148 51
www.anchor-diagnostics.com

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