



ANCHOR
OROV PCR Kit

Instructions for Use
ANCHOR OROV PCR Kit

RUO

Qualitative


Real-Time PCR Kit

For Research Use Only


RUO For Research Use Only


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 -30°C to -15°C

 **ANCHOR** Diagnostics GmbH
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D-22529 Hamburg

compatible with

QuantStudio 5 (Applied Biosystems)

QuantStudio 5 Dx (Applied Biosystems)

LightCycler 480 II (Roche)

cobas z 480 Analyzer (Roche)

LightCycler PRO (Roche)

CFX Opus 96 Dx (Bio-Rad)

CFX96 Deep Well Dx (Bio-Rad)

Rotor-Gene Q (QIAGEN)

Mic qPCR (Bio Molecular Systems)

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2 ▶ Product Description

The ANCHOR OROV PCR Kit is a real-time PCR technology-based test for the reverse transcription, amplification, qualitative detection of nucleic acid harboring the S-segment of the Oropouche virus (OROV) genome.

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 of OROV specific nucleic acids and the Internal Control in two corresponding detector channels of the real-time PCR instrument.

The Positive Control contains a defined concentration of synthetic RNA bearing the OROV target sequences. It can be used together with the Negative Control 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, reverse transcriptase, magnesium ions, dNTPs, primers and probes) to allow RT-PCR-mediated reverse transcription, amplification and target detection of OROV specific RNA and Internal Control in one reaction set-up.

The Positive Control (PC) and Negative Control (NC RNA) are supplied with the Internal Control (IC RNA) already incorporated (see also section 8.2.1 Master Mix Set-Up).

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

Kit component	Mat. no.	No. of vials	Quantity per vial	Ingredients
Master A OROV	AD03608	4 vials	125 µL	Buffer, bovine serum albumin, polymerase, reverse transcriptase
Master B OROV	AD03609	4 vials	125 µL	Buffer, salt, nucleotides, target- and IC-specific oligonucleotides
IC RNA	AD00022R	1 vial	1000 µL	Buffer, target- and IC-specific synthetic polynucleotides
PC OROV*	AD03605	1 vial	200 µL	Buffer, target- and IC-specific synthetic Polynucleotides
NC RNA*	AD00023R	1 vial	200 µL	Buffer, IC-specific synthetic polynucleotides

***INTERNAL CONTROL INSIDE!**

4 ▶ Storage and Stability

- The ANCHOR OROV PCR Kit is shipped on dry ice and should be stored at -30 to -15°C upon receipt.
- The components are stable until the expiration date stated on the label.
- Do not use components of the kit that have passed their expiration date.
- Store OROV RNA and/or potentially positive material 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 OROV the NC RNA and the IC RNA, thawing and freezing cycles up to 4x are allowed.
- 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 a cooling block.
- Do not store kit components 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 viral target region might result in 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 contamination or impurities.

7 ▶ Warnings and Precautions

- For research use only. Not for use in diagnostic procedures.
- Do not use the reagents, if they are thawed upon receipt.
- Use of this product is limited 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 setup 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 ▶ Nucleic Acid Purification

Purified RNA is the sample input material for the ANCHOR OROV PCR Kit. It has to be ensured by the user that the chosen nucleic acid purification system is compatible with real-time PCR technology. Extract the nucleic acids according to the manufacturer's instructions.


PLEASE NOTE

-  If sample eluates are not directly used for PCR analysis, store eluates at -30°C 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 IC RNA provided with the ANCHOR OROV PCR Kit should be co-purified with the nucleic acid of interest to monitor sample preparation efficiency and quality.

PLEASE NOTE

-  The IC RNA **MUST NOT** be added directly to the sample.


In case of manual addition of lysis buffer to the sample, always add the IC RNA after lysis buffer has been added to the sample. The required volume of IC RNA 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 RNA volume: 20 µL
- Elution buffer per sample: 60 µL → IC RNA volume: 6 µL

PLEASE NOTE

-  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 RNA 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 µL of the IC RNA 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

PLEASE NOTE



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

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

Number of reactions	1	10(+1)*	N**
Master A OROV	5 µL (X)	55 µL	Y µL
Master B OROV	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 RNA to be used as inhibition control only – IC RNA added to Master Mix preparation:

Number of reactions	1	10(+1)*	N**
Master A OROV	5 µL (X)	55 µL	Y µL
Master B OROV	5 µL (X)	55 µL	Y µL
IC RNA	1 µL (X)	11 µL	Y µL
Volume Master Mix	11 µL	121 µL	Z µL

*10 reactions + 10%

** See formula on next page

PLEASE NOTE



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

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

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.

Use the Master Mix directly for PCR reaction set-up. Temporary storage of the Master Mix at +2 to +8°C will impact performance.



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




The Positive Control PC OROV and the Negative Control NC RNA already contain the IC RNA in a ready-to-use concentration. No addition of IC necessary!

If you want to use a Master Mix preparation with added IC RNA (as inhibition control) in combination with the PC and NC RNA, 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


PLEASE NOTE


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

Prepare the PCR reaction mix step by step:

- If previously stored frozen, thaw eluates containing nucleic acid (and IC RNA) 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 RNA.
- Add **10 µL of eluate** or control (PC OROV or NC RNA). **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!

PLEASE NOTE

 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 optimal amplification curve performance.

Master Mix	Eluate / Control	Reaction Mix
10 µL	10 µL	20 µL

8.3 ▶ PCR Cycler Configuration

The ANCHOR OROV PCR Kit has been evaluated in combination with the following different PCR Cycler platforms:

PCR Cycler Platform	Run Time
QuantStudio 5 (Applied Biosystems)	≈ 31 min.
QuantStudio 5 Dx (Applied Biosystems)	≈ 31 min.
LightCycler 480 II (Roche)	≈ 30 min.
cobas z 480 Analyzer (Roche)	≈ 30 min.
LightCycler PRO (Roche)	≈ 30 min.
CFX Opus 96 Dx (Bio-Rad)	≈ 43 min.
CFX 96 Deep Well Dx (Bio-Rad)	≈ 33 min.
Rotor-Gene Q (QIAGEN)	≈ 40 min.
Mic qPCR (Biomolecular Systems)	≈ 31 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 ▶ Temperature Profile

The ANCHOR OROV PCR Kit has been evaluated in combination with the following different PCR Cycler platforms: **QuantStudio 5, QuantStudio 5 Dx, LightCycler 480 II, cobas z 480 Analyzer, LightCycler PRO, CFX96 Deep Well Dx, CFX Opus 96 Dx and Rotor-Gene Q.**

Hold	50°C	120 sec	x 1
Cycling	95°C	1 sec	x 40
	65°C *	2 sec	
	72°C	1 sec	

*Fluorescence acquisition for OROV and IC

Reaction Volume: 20 µL

Temperature cycling profile for **Mic qPCR**:

Hold	50°C	120 sec	x 1
Cycling	95°C	1 sec	x 40
	63°C *	2 sec	
	72°C	1 sec	

*Fluorescence acquisition for OROV and IC

Reaction Volume: 20 µL

8.3.2 ▶ Specific PCR Cycler Settings

The following tables contain PCR cycler-specific recommendations for the basic configuration of the run settings.

For additional information regarding the cycler settings, color compensation, gain optimization settings, etc. do not hesitate to contact us directly (see section 9 Technical Assistance & Contact Information).

QuantStudio 5 RUO / QuantStudio 5 Dx		
Target	OROV	IC
Detection	FAM	HEX
Run Settings		
<ul style="list-style-type: none"> Block Type: 96-Well 0.1-mL Block Experiment Type: Standard Curve, Chemistry: TaqMan® Reagents Run Mode: Fast Plate attributes: Passive Reference - None 		
Consumables:		
<ul style="list-style-type: none"> 96-Well Fast Thermal Cycling Plates (Life Technologies Mat. No. 4346907) MicroAmp™ Optical Adhesive Film (Life Technologies Mat. No. 4311971) 96-Well-PCR-Plate, Skirted, „Low Profile“ white (Starlab Mat. No. E1403-5209) Xtra-Clear Advanced Polyolefin StarSeal (qPCR) (Starlab Mat. No. E2796-9795) 		

LightCycler 480 II / cobas z 480 Analyzer		
Target	OROV	IC
Detection LightCycler 480 II	465/510	533/580
Detection cobas z 480 Analyzer	465/510	540/580
Run Settings:		
<ul style="list-style-type: none"> 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:		
<ul style="list-style-type: none"> 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) 		

LightCycler PRO		
Target	OROV	IC
Detection	FAM (494/523)	HEX (541/565)
Run Settings		
<ul style="list-style-type: none"> Block size: 96 		
Consumables:		
<ul style="list-style-type: none"> 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) 		

CFX96 Deep Well Dx / CFX Opus 96 Dx		
Target	OROV	IC
Detection	FAM	HEX
Run Settings: n.a.		
Consumables:		
<ul style="list-style-type: none"> Hard Shell 96-well PCR Plate, white (Mat. No. HSP9655) Optical flat 8 Cap Strip for 0.2 mL (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) 96-Well-PCR-Plate, Skirted, "Low Profile", white (Starlab, E1403-5209) Xtra-Clear Advanced Polyolefin StarSeal (qPCR) (Starlab, E2796-9795) 		

Rotor-Gene Q		
Target	OROV	IC
Detection	Green	Yellow
Run Settings		
<ul style="list-style-type: none"> Use 72-Well Rotor Perform Auto-Gain optimization before 1st acquisition. 		
Consumables:		
<ul style="list-style-type: none"> Strip Tubes and Caps, 0.1 mL (QIAGEN Mat. No. 981103) 		

Mic qPCR		
Target	OROV	IC
Detection	Green	Yellow
Run Settings		
<ul style="list-style-type: none"> Temperature Control: Standard TAQ 		
Consumables:		
<ul style="list-style-type: none"> Mic Tubes and Caps (Mat. No.68MIC-60653) 		

8.4 ▶ Data Analysis

The following tables contain 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 (e.g., for threshold settings). 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).

QuantStudio™ 5 RUO/ QuantStudio 5 Dx
Analysis Settings (all channels):
<ul style="list-style-type: none"> Plot Type: ΔR_n vs Cycle Graph Type: Linear Auto Baseline

LightCycler® 480 II and cobas z 480 Analyzer
Analysis Settings:
<ul style="list-style-type: none"> Abs Quant/Fit Points Color Comp (off) Mean High Confidence

LightCycler PRO
Analysis Settings: <ul style="list-style-type: none"> ▪ Qualitative Analysis ▪ Co compensation (on) ▪ Mean

CFX96 Deep Well Dx / CFX Opus 96 Dx
Analysis Settings (all channels): <ul style="list-style-type: none"> ▪ Baseline Subtracted Curve Fit ▪ Apply Fluorescence Drift Correction ▪ C(t) Determination Mode: Single Threshold ▪ Baseline Cycles: Auto Calculated

Rotor-Gene Q
Analysis Settings (all channels): <ul style="list-style-type: none"> ▪ Quantitation ▪ Linear Scale ▪ Dynamic Tube ON

Mic qPCR
Analysis Settings (all channels): <ul style="list-style-type: none"> ▪ Graph Type: Linear ▪ Method: Dynamic ▪ Ignore Cycles Before: 3 ▪ Threshold Start: 1 ▪ Exclusion: None

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:

Control	OROV	IC
PC OROV	+	+
NC RNA	-	+

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

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

Qualitative result	OROV	IC
OROV RNA positive	+	+/-
OROV RNA negative	-	+
Invalid	-	-

A positive result for OROV RNA 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 RNA 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 or water, 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 OROV PCR Kit.















9 ▶ Technical Assistance & Contact Information

For any questions, or if you identify difficulties using our products do not hesitate to contact us:

phone: +49 40 52 471 62 0

email: support@anchor-diagnostics.com

10 ▶ Symbols

-  Component in Kit
-  Volume per vial
-  Batch code
-  Number of vials
-  Quick Guide - Catalog number and version
-  Product - Catalog number
-  For research use only
-  Unique Device Identifier
-  Catalog number and version
Consult Instructions for Use
-  Important Note
-  Use by
-  Contains sufficient reagents for <N> tests
-  Temperature limits for storage
-  Manufacturer



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