ANCHOR

Viral Respiratory Multiplex PCR Kit

Instructions for Use

Anchor Viral Respiratory Multiplex PCR Kit

RUO

Qualitative Real-Time PCR Kit

for Research Use Only

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REF AD03000

100

HB AD03090-UK - 14.11.2024

QG AD03091-UK - 14.11.2024

-30°C to -15°C

ANCHOR Diagnostics GmbH
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D-22529 Hamburg

compatible with

CFX 96 / Opus (Bio-Rad)

QuantStudio 5 RUO / Dx (Applied Biosystems)

LightCycler PRO (Roche)

1 Contents

2 Product Description

The Anchor Viral Respiratory Multiplex PCR Kit is a Real-Time PCR technology based test for the amplification, detection and differentiation of nucleic acids from Respiratory Syncytial Virus (RSV) A and B, Influenza A, Influenza B and SARS-CoV-2.
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 RSV, Influenza A, Influneza B and SARS-CoV-2 nucleic acids and the Internal Control (IC) in five corresponding detector channels of the Real Time PCR instrument. The Positive Control contains a defined concentration of synthetic RNA bearing the target sequences of RSV, Influenza A, Influenza B and SARS-CoV-2.. They can be used together with the Negative Control RNA 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 reverse transcription, magnesium ions, dNTPs, primers and probes) to allow RT-PCR mediated amplification and target detection of RSV, Influenza A, Influenza B and SARS-CoV-2 specific RNA and Internal Control in one reaction setup.
The PC (Positive Control) and NC (Negative Control) RNA 2 are supplied with the IC (Internal Control) RNA 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 RSV + Inf A/B + SARS-CoV-2	Master B RSV + Inf A/B + SARS-CoV-2	IC RNA 2	! PC RSV + Inf A/B + SARS-CoV-2	! NC RNA 2
AD03008	AD03009	A0023R	AD03005	A0033R
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, IC-specific synthetic Polynucleotide	Contains: Buffer substance, IC-specific synthetic Polynucleotide

! INTERNAL CONTROL INSIDE !

4 Storage and Stability

- The Anchor Viral Respiratory Multiplex PCR Kit is shipped on dry ice and should be stored at –30 to –15°C upon receipt.
- Store RSV, Influenza A, Influenza B as well as SARS-CoV-2 RNA-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 RNA 2 and the IC RNA 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 Resp. MPX 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 RNA is the sample input material for the Anchor Viral Respiratory Multiplex 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 RNA 2 provided with the Anchor Viral Respiratory Multiplex PCR Kit should be co-purified with the nucleic acid of interest to monitor sample preparation efficiency and quality.

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

Always add the Internal Control RNA 2 after lysis buffer has been added to the sample.
The required volume of Internal Control RNA 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 RNA 2 volume: 20 µL
- Elution buffer per sample: 60 µL -> IC RNA 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 RNA 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 1.0 µL of the IC RNA 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 RNA 2 present in sample eluates – NO IC RNA 2 added to Master Mix preparation:

Number of reactions	1	10(+1)*	N**
Master A Resp. MPX	5 µL (X)	55 µL	Y µL
Master B Resp. MPX	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 2 to be used as inhibition control only – IC RNA 2 added to Master Mix preparation:

Number of reactions	1	10(+1)*	N**
Master A Resp. MPX	5 µL (X)	55 µL	Y µL
Master Resp. MPX	5 µL (X)	55 µL	Y µL
IC RNA 2	1 µL (X)	11 µL	Y µL
Volume Master Mix	11 µL	121 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:

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

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.

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

ⓘ The Positive Control Resp. MPX and the Negative Control RNA 2 already contain the IC RNA 2 in a ready-to-use concentration. No addition of IC necessary!

If you want to use a Master Mix preparation with added IC RNA 2 (as inhibition control) in combination with the Positive Control Resp. MPX and NC RNA 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 RNA 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 RNA 2.
- Add **10 µL of eluate** or control (Positive Control Resp. MPX or Negative Control RNA 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		10 µL		20 µL

8.3 PCR Cycler Configuration

The Anchor Viral Respiratory Muliplex PCR Kit has been evaluated in combination with the following different PCR Cycler platforms:

PCR Cycler Platform	Run Time
QuantStudio 5 (Applied Biosystems)	≈ 30 min.
LightCycler PRO	≈ 32 min.
CFX96 (Bio-Rad)	≈ 35 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 PRO and CFX96:**

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

* Fluorescence acquisition

Reaction Volume: 20 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® PRO	SARS CoV-2	494/523	**Run Settings:** ▪ Block size: 96 **Consumables:** ▪ LC480 Multiwell Plate 96, white (Roche Mat. No. 04729692001) ▪ LC480 Sealing Foil (Roche Mat. No. 04729757001)
	IC RNA 2	541/565	
	Influenza A	574/601	
	Influenza B	657/675	
	RSV A/B	687/725	
Bio-Rad CFX96 / Opus	SARS CoV-2	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 RNA 2	HEX	
	Influenza A	TEXAS RED	
	Influenza B	Cy5	
	RSV A/B	Cy5.5	

Instrument	Target	Detection channel	Recommendations / Requirements
QuantStudio™ 5 RUO / Dx	SARS CoV-2	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 RNA 2	TEXAS RED	
	Influenza A	HEX	
	Influenza B	Cy5	
	RSV A/B	IRD700	

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® PRO	**Analysis Settings:** ▪ Baseline divided ▪ Color Comp (on) ▪ Mean ▪ Positive target confirmation without internal control
Bio-Rad CFX96 / Opus	**Analysis Settings (all channels):** ▪ Baseline Substracted Curve Fit ▪ C(t) Determination Mode: Single Threshold ▪ Baseline Threshold: - Baseline Cycles: Auto Calculated
QuantStudio™ 5 RUO / Dx	**Analysis Settings (all channels):** ▪ Plot Type: ΔRn vs Cycle ▪ Graph Type: Linear ▪ Baseline Start/End: 3/15

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	SARS CoV-2	Influenza A	Influenza B	RSV A/B	IC
PC Resp. MPX	+	+	+	+	+
NC RNA 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.

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

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

Result	SARS CoV-2	Influenza A	Influenza B	RSV A/B	IC
SARS CoV-2 RNA positive	+	-	-	-	+/-
Influenza A RNA positive	-	+	-	-	+/-
Influenza B RNA positive	-	-	+	-	+/-
RSV A/B RNA positive	-	-	-	+	+/-
SARS CoV-2, Influenza A, Influenza B, RSV A/B RNA positive	+	+	+	+	+/-
SARS CoV-2, Influenza A, Influenza B, RSV A/B RNA negative	-	-	-	-	+
Invalid	-	-	-	-	-

A positive result forSARS CoV-2 and/or Influenza A and/or Influenza B and/or RSV A/B 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 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 Viral Respiratory Multiplex 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
HB	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

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Version 11/2024