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ANCHOR

**Viral Respiratory Multiplex
PCR Kit**

Instructions for Use
ANCHOR Viral Respiratory Multiplex PCR Kit

RUO


Qualitative Real-Time RT-PCR Kit

For Research Use Only

RUO For Research Use Only


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 AD03090-EN, 2025-11-19

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 -30°C to -15°C

 **ANCHOR** Diagnostics GmbH
Grandweg 64
D-22529 Hamburg

compatible with

QuantStudio 5 / QuantStudio 5 Dx (Applied Biosystems)

LightCycler PRO (Roche)

CFX 96 Deep Well Dx / CFX Opus 96 Dx (Bio-Rad)

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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 (M gene), Influenza A (MP gene), Influenza B (NP gene), and SARS-CoV-2 (S gene & N gene).

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 SARS-CoV-2, Influenza A, Influenza B, and RSV A/B 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 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 are supplied with the IC (Internal Control) RNA already incorporated (see also section 8.2.1 Master Mix Set-Up).

Kit component	Mat. no.	No. of vials	Quantity	Ingredients
Master A VReX	AD03008	4 vials	4x 125 µL	Buffer, bovine serum albumin, polymerase
Master B VReX	AD03009	4 vials	4x 125 µL	Buffer, salt, nucleotides, target- and IC-specific oligonucleotides
IC RNA	AD00022R	1 vial	1000 µL	Buffer, IC-specific synthetic polynucleotide
PC VReX*	AD03005	1 vial each	1x 200 µL	Buffer, target- and IC-specific synthetic polynucleotide, IC-specific synthetic polynucleotide
NC RNA*	AD00023R	1 vial	200 µL	Buffer, 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 -5°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 VReX, the NC RNA and the IC RNA, 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 VReX 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 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 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 ▶ Nucleic Acid Purification

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.


PLEASE NOTE

-  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 labeled clearly and unambiguously to avoid a mix-up of samples.

8.1.2 ▶ Internal Control

The Internal Control RNA 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.

PLEASE NOTE

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

Always add the Internal Control RNA after lysis buffer has been added to the sample.


The required volume of Internal Control 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 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 present in sample eluates – NO IC RNA added to Master Mix preparation:

Number of reactions	1	10(+1)*	N**
Master A VRex	5 µL (X)	55 µL	Y µL
Master B VRex	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 VRex	5 µL (X)	55 µL	Y µL
Master A VRex	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 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 VRex and the Negative Control 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 Positive Control VRex 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

i 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) 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 (Positive Control VReX or Negative Control 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

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

i 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 for achieving optimal amplification curve performance.

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

8.3 ▶ PCR Cycler Configuration

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

PCR Cycler Platform	Run Time
QuantStudio 5 / QuantStudio 5 Dx (Applied Biosystems)	≈ 30 min.
LightCycler PRO (Roche)	≈ 32 min.
CFX 96 Deep Well Dx / CFX Opus 96 Dx (Bio-Rad)	≈ 35 / 45 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, QuantStudio 5 Dx, LightCycler PRO, CFX96, and CFX Opus 96:**

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

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

QuantStudio 5 / QuantStudio 5 Dx					
Target	SARS CoV-2	IC RNA	Influenza A	Influenza B	RSV A/B
Detection	FAM	HEX	TEXAS RED	Cy5	Cy5.5
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. E14035209) Xtra-Clear Advanced Polyolefin StarSeal (qPCR) (Starlab Mat. No. E2796-9795) 					

LightCycler PRO					
Target	SARS CoV-2	IC RNA	Influenza A	Influenza B	RSV A/B
Detection	494/523	541/565	574/601	657/675	687/725
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 Sealing Foil (Roche Mat. No. 04729757001) 					

CFX96 / CFX Opus 96					
Target	SARS CoV-2	IC RNA	Influenza A	Influenza B	RSV A/B
Detection	FAM	HEX	TEXAS RED	Cy5	Cy5.5
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) 					

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. 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 / QuantStudio 5 Dx
Analysis Settings (all channels): <ul style="list-style-type: none"> Plot Type: ΔR_n vs Cycle Graph Type: Linear Baseline Start/End: 3/15

LightCycler PRO
Analysis Settings: <ul style="list-style-type: none"> Baseline divided Color Comp (on) Mean Positive target confirmation without internal control

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

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	SARS CoV-2	Influenza A	Influenza B	RSV A/B	IC
PC VReX	+	+	+	+	+
NC RNA	-	-	-	-	+

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	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 for SARS 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 52 471 62 0

email: support@anchor-diagnostics.com

10 ▶ Symbols

COMP Component in Kit

CONT Volume per vial

LOT Batch code


NUM Number of vials

QG Quick Guide - Catalog number and version

REF Product - Catalog number


RUO For research use only

UDI Unique Device Identifier

 Catalog number and version
Consult Instructions for Use

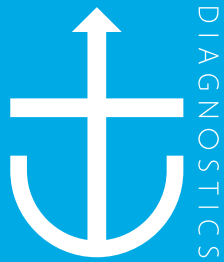
 Important Note

 Use by

 Contains sufficient reagents for <N> tests

 Temperature limits for storage

 Manufacturer



Grandweg 64
22529 Hamburg | Germany
phone: +49 40 52 471 62 0
www.anchor-diagnostics.com