Quick Start Protocol

FBC107-4-1A res4plex *direct* RT-PCR - "Procedure with RNA extraction"

Test for the direct qualitative detection of SARS-CoV-2, Influenza A, B and RSV on a qPCR cycler

Read the entire Instructions for Use (IFU) and follow them carefully before performing the test.
Deviations from the given test protocol can lead to incorrect results. Good laboratory practice should be followed during the test.

Intended Use

The res4plex *direct* RT-PCR test is an *in vitro* real-time reverse transcription polymerase chain reaction (rRT-PCR) test for the qualitative detection of viral RNA of the pathogens SARS-CoV-2, Influenza A, Influenza B, and Respiratory Syncytial Virus (RSV A and RSV B) from respiratory tract specimens (nasopharyngeal or oropharyngeal swabs) from individuals suspected of having a serious viral cold. The results support the differential diagnosis of infections with SARS-CoV-2, Influenza viruses and RSV. The test is intended for use in qualified laboratories by personnel trained in molecular diagnostic techniques.

Package Content

Vials with solution A (blue lid; 1.5 mL) and solution B (yellow lid; 100 µL), each for one microtiter plate (96 well; not included), Positive Control PC (green lid; 13 µL; optional), Negative Control NC (colourless lid; 100 µL; optional), Instructions for Use.

Notes before starting

The starting material for the res4plex *direct* RT-PCR test is 10 µL/reaction RNA isolated from biological specimens (respiratory samples). Appropriate RNA extraction needs to be conducted according to the manufacturer's instructions. RNA extraction reagents are not part of the res4plex *direct* RT-PCR test. One Positive and one Negative Control should be included in each PCR run.

Material provided by user

- RNA isolation kit
- Adequate pipettes and sterile filter-tips for PCR testing (DNase/RNase-free)
- qPCR microtiter plate or reaction tubes; table centrifuge
- qPCR instrument (e.g. LightCycler 480; Biorad CFX)

Test procedure with RNA extraction *(for „Procedure for direct testing" turn page)*

1. **Thaw all reagents** completely and **keep them cool (+2 °C to +8 °C)** directly before starting the test, use within 4 hours.
2. Add **100 µl of solution B to the lysis buffer** per extraction run to be processed in a 96-well plate.
3. Perform RNA extraction according to your laborartory´s standard procedure.
4. Pipette **15 µL/well** of **solution A into** the PCR microtiter plate/reaction tubes.
5. **Add 10 µL/well of eluate** from RNA extraction **and 10 µL Positive Control** as well as **Negative Control** in the respective wells**.**
6. Close the microtiter plate with an adhesive optical film or the reaction tubes with the lids provided.
7. Briefly centrifuge the microtiter plate/reaction tubes, if necessary.
8. Place the filled microtiter plate/reaction tubes in the qPCR cycler. Start program.

Instrument settings

Steps	Temperature [°C]	Time	Number of cycles
Reverse transcription	55	10 min	1x
Initial denaturation	95	2 min	1x
Denaturation	95	5 sec	45x
Amplification/Elongation	63	15 sec	

Channel settings for FBC107-4-1A

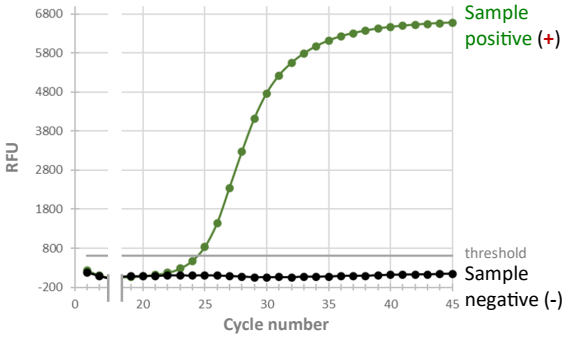
	Flu A	CoV2_N	CoV2_E	Flu B	RSV	IC
Reporter dye	Cyan500	FAM	FAM	HEX	Red 610	Cy5
Colour	blue	green	green	yellow-green	orange	red
Emission [nm]	480	520	520	560	610	670
Quencher	Black Hole Quencher					

Interpretation of test results

Positive samples (+) show a qPCR typical amplification curve that crosses a certain threshold generating the Ct value.
Co-infections of two or more pathogens are possible, but with low probability of occurrence and are therefore not included in the table below.

The results are used to identify SARS-CoV-2, Influenza A, Influenza B and RSV RNA. Positive results are an indication of the presence of the respective virus(es).
A negative result does not rule out the presence of the respective pathogen, as the results depend on correct sampling and a sufficient amount of RNA to be detected.

Flu A	CoV2_N/E	Flu B	RSV	IC	Result	Interpretation
+	-	-	-	+/-	Valid	Influenza A detected.
-	+	-	-	+/-	Valid	SARS-CoV-2 detected.
-	-	+	-	+/-	Valid	Influenza B detected.
-	-	-	+	+/-	Valid	RSV detected.
-	-	-	-	+	Valid	No SARS-CoV-2, Influenza A, Influenza B
-	-	-	-	-	Invalid	The test result can not be evaluated.



Important notes:

All samples of biological origin and used plates/swabs are to be treated as potential carriers of infectious diseases.

When working with chemicals or when handling samples of biological origin, the safety precautions of the laboratory must be observed.

Storage: -25°C … -18°C Usage: +2°C … +8°C

Before performing this test, read the instructions for use to familiarise yourself with the testing procedure.
You can find them on https://frizbiochem.de/downloads/

If you have any questions or problems, please contact service at FRIZ Biochem GmbH (https://frizbiochem.de/get-in-touch/).

REF FBC107-4-1A IVD CE

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Notes before starting

The starting material for the res4plex *direct* RT-PCR test is 10 µL/reaction solubilised patient sample directly used for RT-PCR without any preparatory steps. Optionally, the solubilized sample can be mixed 1:1 with molecular grade water and/or be heated at 95 °C for 5 minutes before it is added to the reaction solution. Patient samples in solutions with chaotropic salts such as guanidinium thiocyanate must not be used. One Positive and one Negative Control should be included in each PCR run.

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Material provided by user

- Adequate pipettes and sterile filter-tips for PCR testing (DNase/RNase-free)
- qPCR microtiter plate or reaction tubes; table centrifuge
- qPCR instrument (e.g. LightCycler 480; Biorad CFX)

Test procedure for direct testing *(for „Procedure with RNA extraction" turn page)*

1. **Thaw all reagents** completely and **keep them cool (+2 °C to +8 °C)** directly before starting the test, use within 4 hours.
2. *Optional: mix sample 1:1 with molecular grade water.*
3. *Optional: heat sample at 95 °C for 5 minutes.*
4. Prepare the reaction solution: **add 15 µL of solution B to the vial of solution A**; mix/shake briefly and centrifuge if necessary. Do not vortex!
5. Pipette **15 µL/well** of the **reaction solution** into the PCR microtiter plate/reaction tubes.
6. **Add 10 µL/well of** a solubilised **patient sample and 10 µL Positive Control** as well as **Negative Control** in the respective wells**.**
7. Close the microtiter plate with an adhesive optical film or the reaction tubes with the lids provided.
8. Briefly centrifuge the microtiter plates or reaction tubes.
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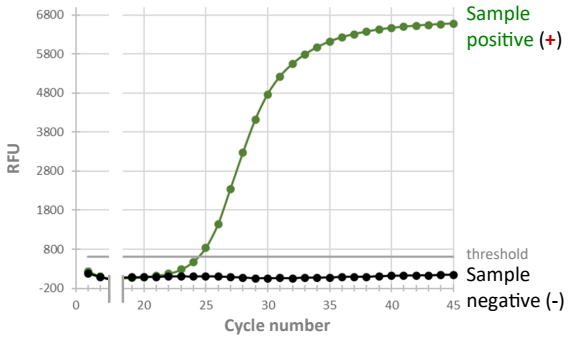
-	-	-	+	+/-	Valid	RSV detected.
-	-	-	-	+	Valid	No SARS-CoV-2, Influenza A, Influenza B
-	-	-	-	-	Invalid	The test result can not be evaluated.



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