FBC107-Bx Quick Start Protocol

res4plex *direct* RT-PCR

Test for the direct qualitative detection of SARS-CoV-2, Influenza A, B and RSV on a **CFX96 or equiv.**

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 Pupose

The res4plex direct RT-PCR test is an assay for in vitro examination of viral RNA in nasal, nasopharyngeal or oral/oropharyngeal swabs to provide information to aid to diagnose patients under suspicion of respiratory diseases: SARS-CoV-2, influenza A, influenza B, and respiratory syncytial virus A/B (RSV A/B). The IVD medical device detects the RNA of the aforementioned pathogens by qualitative measurements based on RT-qPCR and is intended for use in medical laboratories or health institutions by laboratory personnel specifically trained in RT-qPCR and in vitro diagnostic techniques. It has to be used in combination with conventional nucleic acid extraction systems for RNA extraction and RT-qPCR cyclers for detection and analysis.

Package Content for 96 reactions

Solution A (green; 1.1 mL), Internal Control (blue; 400 µL) and Positive Control (red; 20 µL); Quick Start Protocol.

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
- Negative Control
- Adequate pipettes and sterile filter-tips for PCR testing (DNase/RNase-free)
- qPCR microtiter plate or reaction tubes; table centrifuge
- qPCR instrument (e.g. BioRad CFX96 or equiv.)

Test procedure

RNA Extraction

1. Thaw all reagents completely. ☐
2. Add **Internal Control** to the RNA preparation process in accordance to the laboratory´s standard procedure (e.g., add **4µL/sample** to lysis buffer). ☐
3. Perform RNA extraction according to your laboratory's standard procedure. ☐

RT-PCR

4. Pipette **10 µL/well** of **solution A into** the PCR microtiter plate/reaction tubes. ☐
5. **Add 10 µL/well of eluate** from RNA extraction; **add 10 µL Positive Control** per run; add **10 µL Negative Control** per run. ☐
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. ☐
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	61	25 sec	

Channel settings for FBC107-Bx

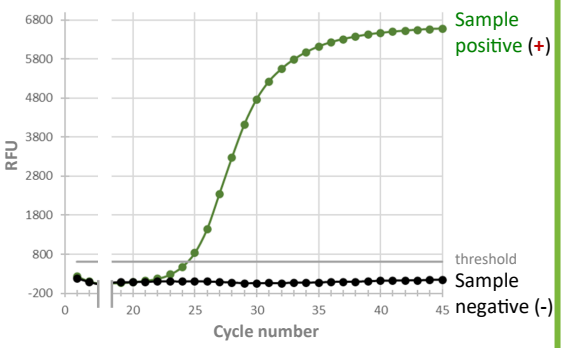
	CoV2_N/E	Flu B	RSV	Flu A	IC
Reporter dye	FAM	HEX	Red 610	Cy5	Cy5.5
Colour	green	yellow-green	orange	red	far-red
Emission [nm]	520	560	610	670	700
Quencher	Black Hole Quencher				

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.

CoV2_N/E	Flu B	RSV	Flu A	IC		
FAM	HEX	Red 610	Cy5	Cy5.5	Result	Interpretation
+	-	-	-	+/-	Valid	SARS-CoV-2 detected.
-	+	-	-	+/-	Valid	Influenza B detected.
-	-	+	-	+/-	Valid	RSV detected.
-	-	-	+	+/-	Valid	Influenza A detected.
-	-	-	-	+	Valid	No SARS-CoV-2, Influenza A, Influenza B or RSV detected.
-	-	-	-	-	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-Bx IVD CE

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