Instructions for Use

In-vitro-Diagnostic

IVD

The res6plex *direct* RT-PCR is a real-time qPCR test for simultaneous *in vitro* detection and differentiation of DNA/RNA from **coronavirus 229E/NL63/OC43/HKU1, metapneumovirus** and **bocavirus.**

Ref.No.:
FBC118

The res6plex *direct* RT-PCR was validated with the Roche LightCycler® 480 II and with the BioRad CFX Opus 96™. In general, Lab *direct* qPCR tests are compatible with other qPCR cyclers (e.g. AJ qTOWER, MIC cycler or LightCycler® 480 I).

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1. Name of the Device

res6plex *direct* RT-PCR

2. Intended Purpose

The res6plex direct RT-PCR is an assay for *in vitro* examination of extracted viral nucleic acids from specimens such as nasal/nasopharyngeal and oral/oropharyngeal swabs to provide information to aid to diagnose symptomatic or asymptomatic patients under suspicion of respiratory tract infections with human Coronavirus (229E/ NL63/ OC43/ HKU1), Metapneumovirus and/or Bocavirus.

The IVD medical device detects the RNA or DNA 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 DNA/RNA extraction and RT-qPCR cyclers for detection and analysis.

3. Pathogen Information

Human coronaviruses (229E, OC43, NL63 and HKU1) are positive-sense, single stranded RNA viruses belonging to the family *Coronaviridae* and subfamily *Orthocoronavirinae*. HCoV-229E and NL63 are classified as α-CoVs, while HCoV-OC43 and HKU1 are classified as β-CoVs. The shape of the coronaviruses is spherical, with a diameter of approximately 125 nm, and they exhibit a solar corona-like appearance due to club-shaped spikes on their surface [1]. These spike glycoproteins mediate receptor binding and membrane fusion for the viral entry [2]. The genome size of the HCoV-229E and HCoV-NL63 are approximately 27.5 kb, whereas those of HCoV-OC43 and HCoV-HKU1 are more than 30 kb.

As pathogens causing upper respiratory tract infections, which are milder compared to SARS-CoV-2 and MERS infections, HCoV-229E, -OC43, -NL63, and -HKU1 are responsible for around 15-30% of common cold cases among adults. HCoV infections are not always limited to the upper respiratory tract and can also lead to infections of the central nervous system (CNS) [3].

Human metapneumovirus (hMPV) is a negative-sense, single-stranded RNA virus belonging to the family *Pneumoviridae* [4,5]. HMPV is genetically similar to RSV, except lacking NS1 and NS2, which are two nonstructural genes of RSV. Metapneumovirus has two major subtypes, hMPV-A and hMPV-B, as well as the sublineages A1, A2a, A2b, B1, B2, which are identified and classified based on the fusion (F) and glycoprotein (G) genes. Since only 2.3% of sequences available in the NCBI GenBank database were complete or partially complete as of November 2020, the genomic epidemiology, genetic diversity and evolution of metapneumovirus remain poorly characterized [4].

According to the epidemiological studies, subtypes of human metapneumovirus cocirculate globally throughout the year, with fluctuations in the predominant subtype [6]. According to World Health Organization (WHO), human metapneumovirus transmission peaks in late winter and spring, coinciding with seasonal influenza and RSV activity [7]. Transmission of metapneumovirus occurs through respiratory droplets generated by coughing and sneezing, direct physical contact such as touching or shaking hands, and contact with contaminated surfaces followed by touching the mouth or nose [8].

Human bocavirus (hBoV) is a nonenveloped, single-stranded DNA virus with a genome size of approximately 5.3 kb. The human bocavirus genome consists of two open reading frames encoding nonstructural proteins and one open reading frame encoding the capsid protein. HBoV1 is the main subtype of bocaviruses causing respiratory infections, particularly among children under the age of 5. Human bocavirus is frequently detected

in coinfections with other respiratory pathogens, such as respiratory syncytial virus, rhinoviruses, adenoviruses (hAdVs), human parainfluenza viruses, influenza A virus, human coronavirus OC43, and SARS-CoV-2 [9].

Bocavirus infections could take place at any time of the year, with the highest rate of incidence in winter and spring. Recent reports indicate that human bocavirus infections peak between December and March in Sweden, whereas peaks in Turkey are observed in November and February. In contrast, the number of bocavirus cases peaks during summer and autumn in China [10].

4. Testing Principle

The res6plex *direct* RT-PCR is a multiplex RT-qPCR test for the detection and differentiation of the following pathogens:

- Coronavirus (229E/NL63/OC43/HKU1)
- Metapneumovirus (A and B)
- Bocavirus (hBoV1)

A separate Internal Control (IC) is added to each sample prior to nucleic acid extraction and serves as control for nucleic acid isolation from the biological specimen as well as for amplification.

The nucleic acid eluate is added to the ready-to-use reaction solution that contains all reagents necessary for RT-qPCR. The analysis can be performed on various RT-qPCR cyclers.

The res6plex *direct* RT-PCR contains primers and probes specific for the targets listed in Table 1 as well as the Internal Control. The probes are each labelled with fluorescent reporter dyes and a second dye that serves as a quencher and suppresses the fluorescence signals of intact probes.

The analysis is performed by determining Ct (cycle threshold) values. The Ct value describes the cycle in which the signal rises above a certain threshold for the first time. The more target copies are present in the sample, the lower the Ct value.

Table 1: Analytes and target genes of res6plex direct RT-PCR

ANALYTE*	GENE	REGION (NUCLEOTIDES)
Coronavirus 229E*	*N*	26200 - 26290
Coronavirus NL63*	*N*	26780 - 26880
Coronavirus OC43*	*N*	29320 - 29400
Coronavirus HKU1*	*N*	28570 - 28655
Metapneumovirus	*F*	3605 - 3715
Bocavirus	*NS1*	1610 - 1820

* *Coronavirus 229E, NL63, OC43 and HKU1 are detected in the same channel and therefore cannot be differentiated.*

5. Package Content

Each kit contains the following vials which are sufficient for 96 reactions (see Table 2).

Table 2: Package content of res6plex direct RT-PCR

MATERIAL	LID COLOUR	#VIALS; VOLUME	#RXNS.	COMMENT	ACTIVE INGREDIENT(S)
Solution A	green	1x; 1050 µL	96	Reaction mix	Polymerase (approx. 0.2 U/µl); reverse transcriptase, primers and probes (approx. 150-200 nM)
Internal Control	blue	1x; 400 µL	96	Artificial nucleic acid target	Mix of RNA (approx. 10^7 copies/µl) and plasmid DNA (approx. 1 pg/µl)
Positive Control	red	1x; 50 µL	4	Nucleic acid of target analytes	RNA with target regions of Coronavirus and Metapneumovirus, DNA plasmid with target region of Bocavirus (each approx. 10^4 copies /µl and 0.1 pg/ µl)

6. Configuration

The res6plex *direct* RT-PCR is available in one configuration, which is suitable for use on a qPCR instrument with at least 4 detection channels (FAM/HEX/Red610/Cy5).

7. Additional Equipment and Reagents (not provided)

- RT-qPCR cycler (with at least 4 detection channels)
- Disposable protective gloves, powder-free
- PCR reaction tubes/microtiter plate plus lids/adhesive optical film
- Pipettes
- Sterile filter-tips for PCR testing (DNA/RNA-free)
- Table centrifuge
- DNA/RNA extraction kit (QIAamp DNA Mini Kit Qiagen N.V. or similar devices)
- Negative Control (no-template control, molecular grade water, or any other negative control according to the laboratory´s standard procedure)

8. Transport, Storage and Stability

The res6plex *direct* RT-PCR is shipped on dry ice. All components must be stored at -25 °C to -18 °C in the dark immediately after receipt. All reagents must be kept at +2 °C to +8 °C during procedure steps and used within 8 hours. Exposure to light should be avoided. You may thaw and refreeze the kit components up to 4 times. The package bears an expiry date, after which no quality guarantee can be given.

9. Warnings, Safety Precautions and Additional Information Access

The res6plex *direct* RT-PCR is intended for *in vitro* diagnostic use only. The test should only be performed by personnel trained in molecular diagnostic techniques. If the user makes changes to the product or the application instructions, results may not correlate with the intended purpose.

All serious incidents relating to the kit must be notified to the manufacturer and the national competent authority of the EU Member State where the laboratory and/or patient is located.

- Before performing the test, read the entire instructions for use and follow them carefully. Deviations from the given test protocols can lead to invalid results.
- All patient samples must be treated as potentially infectious material.
- Discard sample and assay waste that was in contact with patient material according to your local, regional, or national safety regulations.
- The diagnostic sensitivity of the res6plex *direct* RT-PCR is dependent on the DNA/RNA extraction method used to isolate DNA/RNA from biological specimens. It is the responsibility of the user to qualify the extraction methods used.
- Do not use the test beyond the expiration date.
- Do not use the test with opened or damaged packaging.
- Protect reagents from heat, moisture, and light.
- Do not replace or mix the reagents with reagents from other batches or other chemicals.
- Avoid contamination of the test by microorganisms and nucleases (DNases/RNases).
- Any carry-over of samples during handling and processing of the test may result in false positive test results.
- Use separated and segregated working areas for (1) sample preparation, (2) reaction setup and (3) amplification/detection activities. The workflow in the laboratory should proceed in unidirectional manner.
- Always wear disposable gloves in each area and change them before entering a different area.
- The test kits are intended for single use and must not be reused after performing RT-qPCR.
- If contamination of the RT-qPCR cycler is suspected, cleaning and maintenance must be carried out according to the system´s manual.
- In the event of failure of the controls (see chapter 14/Results), the test result cannot be evaluated and a repeat run should be considered.
- Safety Data Sheets (SDS) are available for download via https://frizbiochem.de/downloads/

10. Specimen Collection, Handling, Transport, and Storage

Proper sample collection, storage and transport are critical for PCR analyses in general. Inadequate sample collection, improper sample handling and/or transport can lead to false negative results. Therefore, ensure that:

- The instructions of the medical laboratory and/or the manufacturer of the sample collection system for sample collection, transport and storage are followed.
- The sample collector is instructed to collect the sample in accordance with applicable national laws, regulatory requirements and the instructions for use of the sampling tool.

11. Important Points before Starting

This test is intended for use with samples derived from nasal/nasopharyngeal and oral/oropharyngeal swabs. Other specimen types may cause invalid results.

In every RT-qPCR run one Positive Control and one Negative Control should be included. The Positive Control consists of nucleic acids of respective target pathogens. The Negative Control (e.g. molecular grade water) must be provided by the user. A failed Positive or Negative Control will invalidate the RT-qPCR run and the results must not be reported.

Appropriate nucleic acid extraction methods must be conducted prior to using this assay. Nucleic acid extraction reagents are not part of the res6plex *direct* RT-PCR. Performance evaluation studies have been conducted using the QIAamp DNA Mini Kit (Qiagen N.V.) and Carrier RNA (Qiagen N.V.) for nucleic acid extraction.

12. Test Procedure

All components must be stored at -25 °C to -18 °C in the dark. All reagents must be kept at +2 °C to +8 °C during procedure steps and used within 8 hours.

Table 3: Test procedure of res6plex direct RT-PCR

TEST PROCEDURE	
Sample preparation	
1	Thaw Internal Control only.
2	Add Internal Control to the nucleic acid extraction in accordance with the laboratory´s standard procedure (e.g., add 4µL/sample to lysis buffer; add one additional water sample and use as Negative Control).
3	Put the Internal Control back in the kit box in the deep freezer.
4	Perform nucleic acid extraction according to your laboratory's standard procedure.
RT-qPCR	
5	Thaw Solution A and Positive Control only.
6	Pipette 10 µL/well of Solution A into the PCR microtiter plate/reaction tubes. (If IC is not added to sample during extraction, 0.1 µl IC/sample could be added to Solution A).
7	Add 10 µL/well of eluate from nucleic acid extraction; add 10 µL Positive Control per run; add 10 µL Negative Control per run.
8	Put Solution A and the Positive Control back in the kit box in the deep freezer.
9	Close the microtiter plate with an adhesive optical film or the reaction tubes with the lids provided.
10	Briefly centrifuge the microtiter plate or reaction tubes.
11	Place the filled plate/reaction tubes in the RT-qPCR cycler.
12	Start program.

13. Instrument Settings

The following thermal profile is to be used (Table 4). Channel settings depend on the RT-qPCR cycler used and should be checked before starting the analysis (Table 5). Make sure to activate the detection mode of your RT-qPCR cycler before starting the analysis.

Table 4: Instrument settings of res6plex direct *RT-PCR**

STEPS	TEMPERATURE [°C]	TIME	ACQUISITION	#CYCLES
Initial step	55	5 min	-	1
Denaturation	95	3 min	-	1
Amplification	95	5 sec	-	45
	60	15 sec	on	
	72	15 sec	-	

**If required, the amplification step can be adapted to a two-step cycling protocol by combining the two 60°C/15sec and 72°C/15sec steps into a single 60°C/30sec step.*

Table 5: Channel settings of res6plex direct RT-PCR (Ref. No.: FBC118)

	Coronavirus (229E/NL63/OC43/HKU1)	**Metapenumovirus**	**Internal Control**	**Bocavirus**
	N	*F*	artificial NA	*NS1*
Reporter dye	FAM	HEX	Red 610	Cy5
Colour	green	yellow-green	orange	red
Emission [nm]	520	560	610	670
Quencher	Black Hole Quencher			

The res6plex *direct* RT-PCR was validated with the BioRad CFX Opus 96™ and Roche LightCycler® 480 II. In general, FRIZ Lab *direct* qPCR tests are compatible with many RT-qPCR cyclers. FBC118 is suitable for RT-qPCR cyclers with at least the four channels: FAM, HEX, Red 610, and Cy5.

14.Results

Positive samples show a RT-qPCR typical amplification curve that crosses a certain threshold generating the Ct value (see Figure 1).

A positive signal is characterized by a sigmoidal curve exhibiting both a baseline phase and an exponential phase. Signals that do not exhibit this curve pattern, despite having a Ct value, are considered negative. The threshold shall be set within the exponential phase. It should be noted that in the case of low positive samples, the plateau phase might not be visible due to the cut off after 45 cycles. Thus, the presence of a plateau phase is not an essential requirement for defining a positive signal in such cases.

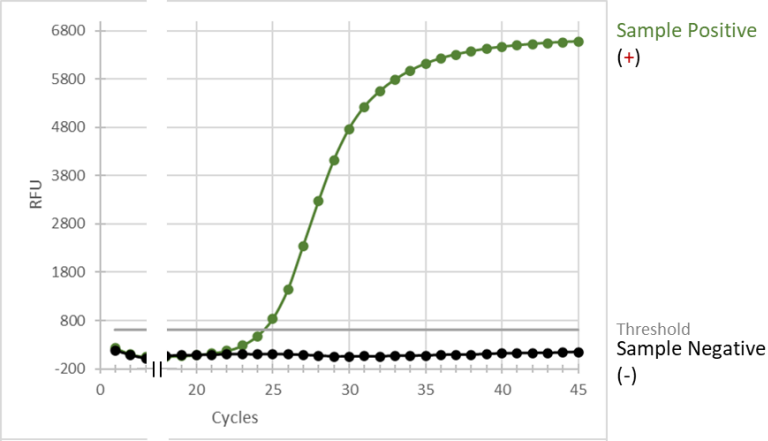


Figure 1: Exemplary amplification curves

- The Negative Control must not show amplification curves in all channels except for the IC channel.
- The Positive Control must show amplification curves in all target channels. If the IC is directly included in Solution A, an amplification curve must also be observed in the IC channel of the Positive Control. The Ct value of the Positive Control must be < 36. A Positive Control with a higher Ct value indicates procedural problems.
- The IC should show a positive amplification curve in both positive and negative samples.

For distribution of results within the channels see Table 6.

Table 6: Results of res6plex direct RT-PCR (Ref. No.: FBC118)

Coronavirus (229E/NL63/OC43/HKU1)	**Metapneumovirus**	**Internal Control**	**Bocavirus**		
FAM	**HEX**	**Red 610**	**Cy5**	**Result**	**Interpretation**
+	–	+	–	Valid	Coronavirus (229E/NL63/OC43/HKU1) detected.
–	+	+	–	Valid	Metapneumovirus detected.
–	–	+	+	Valid	Bocavirus detected.
–	–	+	–	Valid	No Coronavirus, Metapneumovirus, or Bocavirus detected.
+/–	+/–	–	+/–	Invalid	The test result cannot be evaluated.

During the analysis of a res6plex *direct* RT-PCR test with BioRad CFX Opus 96™, activation of ´´Fluorescence Drift Correction´´ is recommended. ´´Fluorescence Drift Correction´´ could be activated through the following steps:

Settings →Baseline Setting → Apply Fluorescence Drift Correction

15.Limitations of the Method

The results support the differential diagnosis of infections with coronavirus 229E/NL63/OC43/HKU1, metapneumovirus and/ or bocavirus.

Positive results indicate the presence of the respective pathogen, but do not exclude a co-infection with other pathogens.

A negative result does not exclude the presence of coronavirus 229E/NL63/OC43/HKU1, metapneumovirus and/or bocavirus as results depend on correct sampling, the absence of inhibitors and sufficient DNA/RNA to

be detected. Invalid results may be obtained if the sample contains inhibitors that prevent lysis, extraction, amplification or detection of the target nucleic acids.

Test results should always be seen in the context of the clinical findings and should not be used as the only basis for diagnosis, treatment, or other patient management decisions. Therapeutic consequences of the diagnostic results must be drawn in relation to the clinical findings by an appropriate healthcare professional.

The detection of analyte target does not mean that they are the causative agents of clinical symptoms.

The res6plex *direct* RT-PCR could detect the presence of the respective nucleic acid of the target pathogen although the clinical symptoms are not observed anymore or the patient is recovered from the diseases state of the target pathogen.

Mutations or polymorphisms in primer and probe binding regions can interfere with the detection of new variants that may result in false negative results.

High concentrations of blood in the specimen (> 1% (v/v)) may inhibit the RT-qPCR and a false-negative result could occur.

The detection of a target analyte does not exclude the possibility of a co-infection with another target analyte since competitive interference may occur. Competitive interference may occur for metapneumovirus (A, B) and coronavirus (229E, NL63, OC43, HKU1) at low concentration (3x LoD) when concentration of bocavirus is ≥ 10^6 copies/ml.

16.Analytical Performance

16.1 Analytical Sensitivity

The limit of detection (LoD) was determined with serial dilutions of genomic DNA or RNA. The analytes were tested in 12 replicates per concentration on a BioRad CFX Opus 96™. The 95% confidence interval (95 CI) was determined using Logistic Regression (Logit) with the GraphPad Prism 10.0.2 software (see Table 7).

Table 7: Limit of Detection of res6plex direct RT-PCR

ANALYTES	LOD (COPIES/REACTION)	UPPER LIMIT	LOWER LIMIT
Coronavirus 229E	5.7	6.1	5.3
Coronavirus NL63	29.5	34.6	24.7
Coronavirus OC43	30.8	37.4	24.8
Coronavirus HKU1	4.3	6.9	2.4
Metapneumovirus A	13.8	35.8	4.3
Metapneumovirus B	23.1	31.3	15.8
Bocavirus	14.1	29.4	5.8

16.2 Other Analytical Performance Parameters

Other analytical performance data, such as analytical specificity including cross-reactivity, endogenous and exogenous interfering substances, inclusivity and competitive interference as well as precision (repeatability and reproducibility) were performed and demonstrated no impact on assay performance, with all test acceptance criteria met.

17.Diagnostic Performance

In total, 233 nasal/nasopharyngeal and oral/oropharyngeal samples were tested. These samples were extracted using the QIAamp DNA Mini Kit (Qiagen N.V.). Eluates were used for parallel analysis with the res6plex *direct* RT-PCR and a CE certified test from another manufacturer in direct comparison on a Bio-Rad CFX Opus 96™. In the event of a discrepancy between the results, another CE certified test from another manufacturer was used to resolve the discrepancy.

Diagnostic sensitivity and specificity are summarized in the following:

Coronavirus samples

		positive	negative	
res6plex	positive	21	0	Sensitivity: 100.0% (83.9 – 100.0)
	negative	0	212	Specificity: 100.0% (98.3 – 100.0)

Metapneumovirus samples

		positive	negative	
res6plex	positive	9	0	Sensitivity: 100.0% (66.4 – 100.0)
	negative	0	224	Specificity: 100.0% (98.4 – 100.0)

Bocavirus samples

		positive	negative	
res6plex	positive	3	0	Sensitivity: 100.0% (29.2 – 100.0)
	negative	0	230	Specificity: 100.0% (98.4 – 100.0)

CE 0123 This product complies with the requirements of the Regulation (EU) *2017*/746 (*IVDR*) for *in vitro* diagnostic medical devices.

18.Symbols

Please note following symbol descriptions according to EN ISO 15223-1.

Graphic	Title	Description

IVD	In vitro diagnostic medical device	Indicates a medical device that is intended to be used as an in vitro diagnostic medical device.
	Manufacturer	Indicate the medical device manufacturer.
	Temperature limit	Indicates the temperature limits to which the medical device can be safely exposed.
	Use-by date	Indicates the date after which the medical device is not to be used.
eIFU Indicator	Consult instructions for use or consult electronic instructions for use	Indicates the need for the user to consult the instructions for use.
CE	CE marking European Conformity	--
REF	Catalogue number (Reference number)	Indicates the manufacturer's catalogue number so that the medical device can be identified.
Σ	Content sufficient for <n> tests	Indicates the total number of tests that can be performed with the medical device.
LOT	Batch code	Indicates the manufacturer's batch code so that the batch or lot can be identified.
	Keep away from sunlight	Indicates a medical device that needs protection from light sources.
UDI	Unique device identifier	Indicates a carrier that contains unique device identifier information.

Document Version:

FBC118_IFU_EN	
Version 1.0 Date: 2026-07-22	First version
Version 1.1 Date: 2026-10-29	Corrected in the scope of deficiency report 1

19.Technical Assistance and Contact Information

For technical assistance, product support, or questions related to the performance or interpretation of this test, users may contact the manufacturer directly.

Manufacturer:	FRIZ Biochem GmbH
Address:	Floriansbogen 2-4 / 82061 Neuried / Germany
Telephone:	+49 (0) 89 72 44 09 0
Email (Technical Assistance):	support@frizbiochem.de
Email (General Enquiry):	info@frizbiochem.de
Website:	www.frizbiochem.de

Technical support is available during regular business hours, and all inquiries will be handled by qualified personnel. Users are encouraged to contact the manufacturer immediately in the event of suspected malfunction, unexpected results, or any issue requiring expert clarification.

20. References

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FRIZ Biochem GmbH ▫ Floriansbogen 2-4 ▫ 82061 Neuried ▫ Germany

Tel +49 (0) 89 72 44 09 0 ▫ Fax +49 (0) 89 72 44 09 10

info@frizbiochem.de ▫ www.frizbiochem.de