Instructions for Use

In-vitro-Diagnostic

IVD

The res5plex *direct* RT-PCR is a real-time qPCR test for simultaneous *in vitro* detection and differentiation of DNA/RNA from **parainfluenza virus (1/2/3/4), adenovirus** and **enterovirus/rhinovirus.**

Ref.No.:
FBC117

The res5plex *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).

Content

1. Name of the Device

res5plex *direct* RT-PCR

2. Intended Purpose

The res5plex *direct* RT-PCR is an assay for *in vitro* examination of viral nucleic acids in nasal/nasopharyngeal and oral/oropharyngeal swabs to provide information to aid to diagnose symptomatic or asymptomatic patients under suspicion of respiratory tract infections with Parainfluenza virus 1/ 2/ 3/ 4, Adenovirus and/or Enterovirus/Rhinovirus.
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 parainfluenza viruses are single stranded RNA viruses belonging to the *Paramyxoviridae* family. Human Parainfluenza viruses 1 and 3 belong to the genus Respirovirus and human parainfluenza viruses 2 and 4 belong to the genus Rubulavirus. Diameters of parainfluenza viruses vary between 150 and 200 micrometers. The RNA of parainfluenza viruses encodes nucleocapsid protein (NP), phosphoprotein (P), fusion glycoprotein (F), matrix protein (M), hemagglutinin-neuraminidase (HN) glycoprotein and RNA polymerase (L) [1,2].

The transmission of parainfluenza virus usually occurs through the air by coughing and sneezing, physical contact with the infected people or objects followed by physical contact with the mouth, nose or eyes. Human parainfluenza virus circulates anytime of the year although spring, summer and fall are the peak seasons for the transmission of parainfluenza virus [3].

Adenoviruses are double stranded DNA viruses with more than 120 serotypes [4]. The size of adenoviruses is approximately 65-90 nm with a 26-48 kb non-segmented linear double stranded DNA genome, transcribing early (E), intermediate (I) and late (L) regions. The late (L) region encodes proteins involved in capsid formation, DNA encapsulation and maturation of progeny adenovirus virion [4,5].

Adenoviruses mainly cause acute respiratory diseases such as cold, pneumoniae and bronchitis. However, different types of adenoviruses could lead to keratoconjunctivitis epidemica, pharyngoconjunctival fever, follicular conjunctivitis, gastroenteritis, pharyngitis and less frequently, meningoencephalitis. Adenoviruses are transmitted by physical contact, fecal and oral interactions, through water, or contact with the eyes. Adenoviruses are very resistant to the environment, a reason why they can be infectious for weeks at room temperature [6].

Enteroviruses and rhinoviruses are single stranded, positive polarity RNA viruses belonging to the family *Picornaviridae*. More than 110 enteroviruses and 160 rhinoviruses were identified, which were grouped as enterovirus A-L and rhinovirus A-C. Infections of these pathogens could lead to a broad spectrum of illnesses including pneumonia and bronchiolitis, cystic fibrosis, asthma, acute flaccid myelitis (AFM), neonatal sepsis, aseptic meningitis, myocarditis, hand-foot-mouth disease, and encephalitis especially among kids younger than 6 years old [7]. Especially rhinoviruses and enterovirus D68 infections are highly related to respiratory complications [7]. Both could be transmitted through physical contact with infected people or surfaces and touching eyes, nose or mouth as well as contact with fecal samples and through water [8].

4. Testing Principle

The res5plex *direct* RT-PCR is a multiplex RT-qPCR test for the detection and differentiation of the following pathogens:

- Parainfluenza virus 1/2/3/4
- Adenovirus (1, 2, 3, 4, 5, 7, 14, 21, 35 and 55)
- Enterovirus/Rhinovirus

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 res5plex *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 res5plex direct RT-PCR

ANALYTE*	GENE	REGION (NUCLEOTIDES)
Parainfluenza virus 1*	*L*	11660 - 11850
Parainfluenza virus 2*	*HN*	8115 - 8250
Parainfluenza virus 3*	*NP*	626-781
Parainfluenza virus 4*	*N*	650-875
Adenovirus	*Hexon*	18402 - 18590
Enterovirus/Rhinovirus#	*5`UTR*	248 - 451

* *Parainfluenza virus 1,2,3 and 4 are detected in the same channel and therefore cannot be differentiated.*

 # Enterovirus and Rhinovirus 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 res5plex 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 Parainfluenza virus and Rhinovirus, DNA with target region of Adenovirus (each approx. 10^4 copies /µl)

6. Configuration

The res5plex *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 res5plex *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. 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 res5plex *direct* RT-PCR is intended for *in vitro* diagnostic use only. The test should only be performed by personnel trained in molecular laboratory 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 infectious material according to your local, regional, or national safety regulations.
- 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 res5plex *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.

Table 3: Test procedure of res5plex 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 res5plex 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 res5plex direct RT-PCR (Ref. No.: FBC117)

	Parainfluenza virus (1/2/3/4)	**Adenovirus**	**Internal Control**	**Enterovirus/ Rhinovirus**
	L/HN/NP/N	*Hexon*	artificial NA	*5´ UTR*
Reporter dye	FAM	HEX	Red 610	Cy5
Colour	green	yellow-green	orange	red
Emission [nm]	520	560	610	670
Quencher	Black Hole Quencher			

The res5plex *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. FBC117 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.

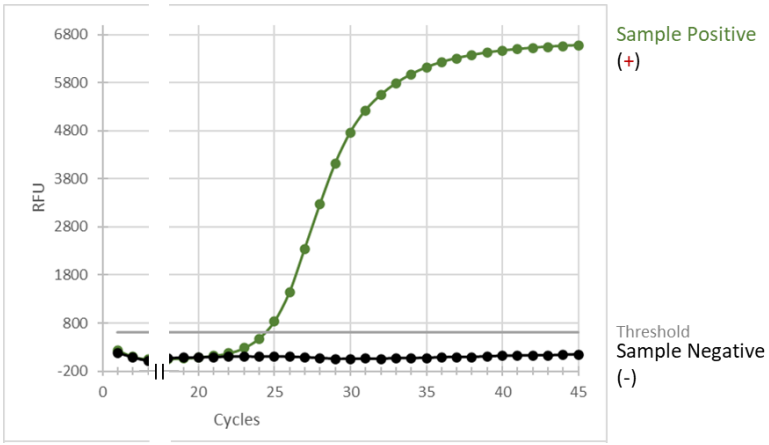

Figure 1: Exemplary amplification curves

- 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 res5plex direct RT-PCR (Ref. No.: FBC117)

Parainfluenza virus 1/2/3/4	Adenovirus	Internal Control	Enterovirus/ Rhinovirus		
FAM	**HEX**	**Red 610**	**Cy5**	**Result**	**Interpretation**
+	–	+	–	Valid	Parainfluenza virus 1/2/3/4 detected.
–	+	+	–	Valid	Adenovirus detected.
–	–	+	+	Valid	Enterovirus/Rhinovirus detected.
–	–	+	–	Valid	No Parainfluenza virus, Adenovirus, Enterovirus, or Rhinovirus detected.
+/–	+/–	–	+/–	Invalid	The test result cannot be evaluated.

During the analysis of a res5plex *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 parainfluenza virus (1/2/3/4), enterovirus/ rhinovirus and/or adenovirus.

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 parainfluenza virus (1/2/3/4), enterovirus/rhinovirus or adenovirus 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 res5plex *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 rhinovirus 14 at low concentration (3x LoD) when concentration of parainfluenza virus 2 and adenovirus 3 is ≥ 10^6 copies/ml. In addition, competitive interference may occur for enterovirus 68 at low concentration (3x LoD) when concentration of adenovirus 3 is ≥ 10^6 DNA 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 res5plex direct RT-PCR

ANALYTES	LOD (COPIES/REACTION)	UPPER LIMIT	LOWER LIMIT
Parainfluenza virus 1	9.3	13.9	5.7
Parainfluenza virus 2	6.2	10.8	3.0
Parainfluenza virus 3	21.7	35.7	12.1
Parainfluenza virus 4	5.6	5.9	5.4
Adenovirus B3	10.9	13.1	8.8
Adenovirus E4	18.1	27.2	11.3
Adenovirus C5	33.2	56.1	17.5
Enterovirus A71	29.7	32.4	27.2
Enterovirus D68	27.2	36.5	19.0
Rhinovirus B14	18.3	23.3	13.8

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 res5plex *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:

Parainfluenza virus samples

		positive	negative	
res5plex	positive	15	0	Sensitivity: 100.0% (78.2 - 100.0)
	negative	0	218	Specificity: 100.0% (98.3 - 100.0)

Adenovirus samples

		positive	negative	
res5plex	positive	9	0	Sensitivity: 100.0% (66.4 - 100.0)
	negative	0	224	Specificity: 100.0% (98.4 - 100.0)

Enterovirus/ Rhinovirus samples

		positive	negative	
res5plex	positive	27	0	Sensitivity: 100.0% (87.2 - 100.0)
	negative	0	206	Specificity: 100.0% (98.2 - 100.0)

The diagnostic sensitivity of the res5plex *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.

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
	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.
	Consult instructions for use or consult electronic instructions for use	Indicates the need for the user to consult the instructions for use.
	CE marking European Conformity	--
	Catalogue number (Reference number)	Indicates the manufacturer's catalogue number so that the medical device can be identified.
	Content sufficient for <n> tests	Indicates the total number of tests that can be performed with the medical device.
	Do not reuse	Indicates a medical device that is intended for one single use only.
	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.
	Unique device identifier	Indicates a carrier that contains unique device identifier information.

Document Version:

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Version 1.0 Date: 2026-07-22	First version

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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