



For *in Vitro* Diagnostic Use

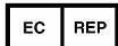
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# AmpliSens<sup>®</sup> Parainfluenza virus-FRT PCR kit

## Instruction Manual

# AmpliSens<sup>®</sup>



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### 1. INTENDED USE.

**AmpliSens® Parainfluenza virus-FRT** PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and differentiation of *Parainfluenza virus* types 1, 2, 3, and 4 RNA in the clinical materials (nasal and throat swabs; sputum; autopsy material) by using real time hybridization-fluorescence detection of amplified products.

### 2. PRINCIPLE OF PCR DETECTION.

*Parainfluenza virus* types 1, 2, 3, 4 detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogenic genome specific region using specific *Parainfluenza virus* primers. In real-time PCR the amplified product is detected using fluorescent dyes. These dyes are usually linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The real-time PCR monitoring of the fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run. **AmpliSens® Parainfluenza virus-FRT** PCR kit is a qualitative test, which contains the Internal Control (IC). It must be used in the isolation procedure in order to control the isolation process of each individual sample and to identify possible reaction inhibition. **AmpliSens® Parainfluenza virus-FRT** PCR kit uses “hot-start”, that is guaranteed by separation of nucleotides and Taq-polymerase by wax layer. Wax melting and reaction mix components occur only at 95 °C, which greatly diminishes frequency of nonspecifically primed reactions.

### 3. CONTENT.

**AmpliSens® Parainfluenza virus-FRT** PCR kit is produced in 1 form:

**AmpliSens® Parainfluenza virus-FRT** PCR kit variant FRT, **REF** R-V51(RG)-CE

**AmpliSens® Parainfluenza virus-FRT** PCR kit, variant FRT includes:

Reagent	Description	Volume (ml)	Quantity
<b>PCR-mix-1-FRT Parainfluenza virus 1/3 (under wax)</b>	colorless clear liquid	0.008	55 tubes of 0.2 ml volume
<b>PCR-mix-1-FRT Parainfluenza virus 2/4 (under wax)</b>	colorless clear liquid	0.008	55 tubes of 0.2 ml volume
<b>PCR-mix-2-FL</b>	colorless clear liquid	0.77	1 tube
<b>Positive Control cDNA Parainfluenza virus type 1 (C+<sub>1</sub>)</b>	colorless clear liquid	0.1	1 tube
<b>Positive Control cDNA Parainfluenza virus type 2 (C+<sub>2</sub>)</b>	colorless clear liquid	0.1	1 tube
<b>Positive Control cDNA Parainfluenza virus type 3 (C+<sub>3</sub>)</b>	colorless clear liquid	0.1	1 tube

<b>Positive Control cDNA Parainfluenza virus type 4 (C+<sub>4</sub>)</b>	colorless clear liquid	0.1	1 tube
<b>Positive Control STI (CS+)</b>	colorless clear liquid	0.1	1 tube
<b>TE-buffer</b>	colorless clear liquid	0.5	1 tube
<b>Negative Control (C-)*</b>	colorless clear liquid	1.2	1 tube
<b>Internal Control STI-rec**</b>	colorless clear liquid	0.12	5 tubes

\* must be used in the isolation procedure as Negative Control of Extraction.

\*\* add 10 µl of Internal Control STI-rec during the RNA isolation procedure directly to the sample/lysis mixture (see “RIBO-sorb” **REF** K2-1-Et-50-CE protocol).

**AmpliSens® Parainfluenza virus-FRT** PCR kit is intended for 55 reactions, including controls.

### 4. ADDITIONAL REQUIREMENTS.

- RNA isolation kit.
- Reverse transcription kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free pipette tips with aerosol barriers (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2 ml reaction tubes.
- PCR box.
- Rotor-Gene™ 3000 or Rotor-Gene™ 6000 (Corbett Research, Australia) Instrument.
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity (for example, “Axygen”, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer with temperature not more than –16°C.
- Waste bin for used tips.

### 5. GENERAL PRECAUTIONS.

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly at room temperature before starting an assay.

- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Specimens should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid sample or reagent contact with the skin, eyes and mucose membranes. If any of these solutions come into contact, rinse immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- Workflow in the laboratory must proceed in a one directional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents to the area in which the previous step was performed.



Some components of this kit contain Sodium Azide as a preservative. Do not use metal tubing for reagent transfer.

## 6. SAMPLING AND HANDLING.



Obtaining samples of biological materials for PCR-analysis, transportation and storage is described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

**AmpliSens® Parainfluenza virus-FRT** PCR kit is intended for analysis of RNA extracted with RNA isolation kits from nasal and throat swabs; sputum; autopsy material.

### 6.1. Sampling.

6.1.1. *Nasal swab sample* is obtained by dry sterile cotton swab. Gently insert the swab through the nostril 2-3 cm deep towards inferior nasal concha. Lower the swab and pass it under inferior nasal concha. Rotate and remove the swab. Place the effective part of the probe into the tube with 500 µl of Transport medium for storage and transportation of respiratory swabs, **REF** 959. Break off the probe and tightly secure the cap.

6.1.2. *Throat swab sample* is obtained by dry sterile cotton swab. Before sampling make patient rinse the mouth with water. Rotate the swab over tonsillar area, palatine arches, and posterior area of the pharynx. Place the effective part of the probe into the tube with Transport medium for storage and transportation of respiratory swabs, **REF** 959. Break off the probe and tightly secure the cap.



It's recommended to combine nasal and throat swab samples in a single tube. For this purpose, place the effective part of both probes into one tube containing 500 µl of transport medium and analyze as a single sample.

6.1.3. *Sputum sample* is collected in a sterile disposable container. Before sampling make patient rinse the mouth with water.

6.1.4. *Autopsy material* is to be placed into a sterile disposable container and frozen or analyzed within 1 hour.

### 6.2. Material treatment.

6.2.1. *Swabs* are used without additional handling.

6.2.2. *Sputum*. Mucolysin, **REF** 180, is additionally required. Perform the treatment according to the manufacturer instructions. Prepared sample (50µl) is used for RNA extraction. The residue of the sample can be frozen for further use.

6.2.3. *Autopsy material* should be homogenized by a sterile porcelain mortar and pestle. After that 10% suspension should be prepared by adding sterile saline solution or phosphate buffer. Then transfer the suspension in a 1.5 ml tube, centrifuge at 10,000 rpm for 30 sec and use supernatant (100 µl) for RNA extraction.

## 7. PROTOCOL.

### 7.1. RNA Isolation

It's recommended to use the following nucleic acid extraction kits:

- "RIBO-sorb", **REF** K2-1-Et-50-CE.



Carry the RNA isolation according to the manufacturer instruction.



Add 100 µl of Negative Control into the tube for Negative Control of Extraction (C-).

### 7.2. Reverse transcription.

It's recommended to use the following kit for complementary DNA (cDNA) synthesis from RNA:

- "REVERTA-L", containing RT-Gmix, **REF** K3-4-50-CE.



Carry the reverse transcription procedure according to the manufacturer instruction.

### 7.3. Preparing the PCR.

Total reaction volume is **25 µl**, the volume of RNA sample is **10 µl**.

#### **Detection of Parainfluenza virus types 1 and 3 RNA (Respirovirus genus).**

##### 7.3.1 Preparing tubes for PCR.

1. Prepare the required number of the tubes with **PCR-mix-1-FRT Parainfluenza virus 1/3** and wax for

amplification of cDNA from clinical and control samples.

- Add **7 µl** of **PCR-mix-2-FL** to the surface of wax layer of each tube ensuring that it does not fall under the wax and mix with PCR-mix-1-FRT *Parainfluenza virus 1/3*.
- Using tips with aerosol barrier add **10 µl** of **cDNA samples** obtained from clinical or control samples at the stage of reverse transcription of RNA.
- Carry the control amplification reactions:

- NCA** - Add **10 µl** of **TE-buffer** to the tube labeled NCA (Negative Control of Amplification).
- C+<sub>1</sub>** - Add **10 µl** of **Positive Control cDNA Parainfluenza virus type 1** to the tube labeled C+<sub>1</sub>.
- C+<sub>3</sub>** - Add **10 µl** of **Positive Control cDNA Parainfluenza virus type 3** to the tube labeled C+<sub>3</sub>.
- CS+** - Add **10 µl** of **Positive Control STI** to the tube labeled CS+.

### 7.3.2. Amplification

- Program the Rotor-Gene™ according to manufacturer's manual and Appendix 1.
- Create a temperature profile on your Rotor-Gene™ instrument as follows:

Table 1

Programming of Rotor-Gene at amplification of *Parainfluenza virus* types 1, 3 cDNA

Step	Temperature, °C	Time	Fluorescence detection	Cycles
Hold	95	5 min	–	1
Cycling 1	95	10 sec	–	10
	54	20 sec	–	
	72	10 sec	–	
Cycling 2	95	10 sec	–	35
	54	20 sec	FAM/Green, JOE/Yellow, ROX/Orange	
	72	10 sec	–	

- Fluorescence detection is on the 2-nd pass (**54 °C**) on FAM/Green, JOE/Yellow and ROX/Orange fluorometer channels.
- Make the adjustment of the fluorescence channel sensitivity according to Appendix 1.

### Detection of *Parainfluenza virus* types 2 and 4 RNA (*Rubulavirus* genus).

#### 7.3.3. Preparing tubes for PCR.

- Prepare the required number of the tubes with **PCR-mix-1-FRT *Parainfluenza virus 2/4*** and wax for amplification of cDNA from clinical and control samples.
- Add **7 µl** of **PCR-mix-2-FL** to the surface of wax layer of each tube ensuring that it does not fall under the wax and mix with PCR-mix-1-FRT *Parainfluenza virus 2/4*.
- Using tips with aerosol barrier add **10 µl** of **cDNA samples** obtained from clinical or control samples at the stage of reverse transcription of RNA.
- Carry the control amplification reactions:

- NCA** - Add **10 µl** of **TE-buffer** to the tube labeled NCA (Negative Control of Amplification)
- C+<sub>2</sub>** - Add **10 µl** of **Positive Control cDNA Parainfluenza virus type 2** to the tube labeled C+<sub>2</sub>
- C+<sub>4</sub>** - Add **10 µl** of **Positive Control cDNA Parainfluenza virus type 4** to the tube labeled C+<sub>4</sub>
- CS+** - Add **10 µl** of **Positive Control STI** to the tube labeled CS+

### 7.3.4. Amplification.

- Program the Rotor-Gene™ according to manufacturer's manual and Appendix 1.
- Create a temperature profile on your Rotor-Gene™ instrument the same as for amplification of *Parainfluenza virus* types 1, 3 cDNA (see Table 1).
- Fluorescence detection is on the 2-nd pass (**54°C**) on FAM/Green, JOE/Yellow and ROX/Orange fluorometer channels.
- Make the adjustment of the fluorescence channel sensitivity according to Appendix 1.



It's allowed to test simultaneously tubes with PCR-mix-1-FRT *Parainfluenza virus 1/3* and PCR-mix-1-FRT *Parainfluenza virus 2/4*.

## 8. DATA ANALYSIS.

### 8.1. Detection of *Parainfluenza virus* types 1 and 3 RNA (*Respirovirus* genus).

Internal Control is detected in the FAM/Green fluorescence channel, *Parainfluenza virus* type 1 is detected on the ROX/Orange fluorescence channel, *Parainfluenza virus* type 3 is detected on the JOE/Yellow fluorescence channel.

See **Appendix 1** for data analysis settings for Rotor-Gene™ 3000 or Rotor-Gene™ 6000.

#### Results interpretation.

The results are interpreted by the software of Rotor-Gene™ 3000 or Rotor-Gene™ 6000 Instrument through the crossing (or not) of fluorescence curve with the threshold line.

Results are considered to be relevant if positive and negative controls of amplification along with negative control of extraction are passed.

Table 2

Results for controls

Control	Stage for control	Ct on channel			Interpretation
		FAM/Green	JOE/Yellow	ROX/Orange	
<b>C-</b>	DNA isolation	Pos (< N*)	Neg	Neg	OK
<b>NCA</b>	Amplification	Neg	Neg	Neg	OK
<b>C+<sub>1</sub></b>	Amplification	Neg	Neg	Pos (< X*)	OK
<b>C+<sub>3</sub></b>	Amplification	Neg	Pos (< Y*)	Neg	OK
<b>CS+</b>	Amplification	Pos (< Z*)	Neg	Neg	OK

\*For N, X, Y, Z values see Appendix 1.

- The sample is considered to be positive for *Parainfluenza virus* type 1** if its Ct value on ROX/Orange channel is less than X. If Ct value is more than X in ROX/Orange channel, the PCR should be repeated

for this sample. If in the second run the result is the same or less than X the sample should be considered to be positive.

2. **The sample is considered to be positive for *Parainfluenza virus type 3*** if its Ct value on JOE/Yellow channel is less than Y. If Ct value is more than Y in JOE/Yellow channel, the PCR should be repeated for this sample. If in the second run, the result is the same or less than Y the sample should be considered to be positive.
3. **The sample is considered to be negative** if Ct values on ROX/Orange and JOE/Yellow are absent, while Ct value on FAM/Green channel doesn't exceed N.

**8.2. Detection of *Parainfluenza virus types 2 and 4* RNA (*Rubulavirus* genus).**

Internal Control is detected in the FAM/Green fluorescence channel, *Parainfluenza virus type 2* is detected on the JOE/Yellow fluorescence channel, *Parainfluenza virus type 4* is detected on the ROX/Orange fluorescence channel.

See **Appendix 1** for data analysis settings for Rotor-Gene™ 3000 or Rotor-Gene™ 6000.

**Results interpretation.**

The results are interpreted with the software of Rotor-Gene™ 3000 or Rotor-Gene™ 6000 Instrument through the presence (or absence) of crossing of fluorescence curve with the threshold line.

Results are accepted to be relevant if positive and negative controls of amplification along with negative control of extraction are passed.

Table 3

Results for controls

Control	Stage for control	Ct in channel			Interpretation
		FAM/Green	JOE/Yellow	ROX/Orange	
C-	DNA isolation	Pos (< N*)	Neg	Neg	OK
NCA	Amplification	Neg	Neg	Neg	OK
C+ <sub>2</sub>	Amplification	Neg	Pos (< Y*)	Neg	OK
C+ <sub>4</sub>	Amplification	Neg	Neg	Pos (< X*)	OK
CS+	Amplification	Pos (< Z*)	Neg	Neg	OK

\*For N, X, Y, Z values see Appendix 1.

1. **The sample is considered to be positive for *Parainfluenza virus type 2*** if its Ct value on JOE/Yellow channel is less than Y. If Ct value is more than Y in JOE/Yellow channel, the PCR should be repeated for this sample. If in the second run the result is the same or less than Y the sample should be considered to be positive.
2. **The sample is considered to be positive for *Parainfluenza virus type 4*** if its Ct value on ROX/Orange channel is less than X. If Ct value is more than X in ROX/Orange channel, the PCR should be repeated for this sample. If in the second run the result is the same or less than X the sample should be considered to be positive.

3. **The sample is considered to be negative** if Ct values on ROX/Orange and JOE/Yellow are absent, while Ct value in FAM/Green channel doesn't exceed N.

**9. TROUBLESHOOTING.**

Results of analysis are not being registered in the following cases:

1. No positive signal with positive controls of PCR can indicate incorrect programming of the temperature profile of the thermocycler, improper configuration of the PCR, inappropriate storing of kit components, or expiration or reagents kit. It is necessary to check programming of the thermocycler (see Table 1) storage conditions and the expiration date of the reagents and repeat PCR once again for all samples.
2. Positive signal detected in negative control C- (in FAM/Green or JOE/Yellow channels) or negative control of amplification NCA (in any channel) indicates the reagents or samples contamination. In such case results of analysis must be considered to be irrelevant. Test analysis must be repeated and measures for detecting of contamination source must be undertaken.
3. If Ct value detected for Internal Control (FAM/Green channel) is more than N, the analysis for this sample should be repeated starting from the stage of RNA extraction.

If you have any further questions or if you encounter problems, please contact our Authorized representative in the European Community.

**10. STABILITY AND STORAGE.**

All components of the **AmpliSens® *Parainfluenza virus-FRT*** PCR kit are to be stored at the temperature between 2 and 8 °C, when not in use. All components of the **AmpliSens® *Parainfluenza virus-FRT*** PCR kit are to be stable until labeled expiration date.

**11. SPECIFICATIONS.**

**11.1. Sensitivity.**

Analytical Sensitivity of **AmpliSens® *Parainfluenza virus-FRT*** PCR kit is not less than 5x10<sup>3</sup> copies/ml.



The claimed analytical features of **AmpliSens® *Parainfluenza virus-FRT*** PCR kit are guaranteed only when additional reagents kits "RIBO-sorb" and "REVERTA-L" (manufactured by Federal State Institution of Science Central Research Institute of Epidemiology) are used.

**11.2. Specificity.**

Specificity of **AmpliSens® *Parainfluenza virus-FRT*** PCR kit is assured by selection of specific primers and probes, as well as the selection of strict reaction conditions. The primers and probes were checked for possible homologies to all in gene banks published sequences by sequence comparison analysis. Specificity of **AmpliSens® *Parainfluenza virus-FRT*** PCR kit was confirmed in laboratory clinical trials.

















## 12. REFERENCES.

1. Handbook "Sampling, transportation, storage of clinical material for PCR diagnostics", developed by Federal State Institution of Science Central Research Institute of Epidemiology of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being, Moscow, 2008.

## 13. QUALITY CONTROL.

In compliance with Federal State Institution of Science "Central Research Institute of Epidemiology" ISO 13485 – certified Quality Management System, each lot of **AmpliSens® Parainfluenza virus-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

## 14. EXPLANATION OF SYMBOLS.

	Manufacturer		Temperature limitation
	Use by		Batch code
	For <i>in Vitro</i> Diagnostic Use		Version
	Catalogue number		Internal Control complex
	Contains sufficient for <n> tests		Authorized representative in the European Community.
	Consult instructions for use		Caution, consult accompanying documents
	For working with Rotor-Gene™ 3000/6000		For working with iQ5, iQ iCycler
	Positive control		Negative control