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For Professional Use Only

AmpliSens® *Parainfluenza virus*-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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TABLE OF CONTENTS

1. INTENDED USE

AmpliSens® *Parainfluenza virus*-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and differentiation of specific fragments of nucleic acids of *Parainfluenza virus* types 1 and 3 (genus *Respirovirus*) and *Parainfluenza virus* types 2 and 4 (genus *Rubulavirus*) in clinical materials (nasal and oropharyngeal swabs, sputum or tracheal aspirate, bronchoalveolar lavage, or bronchial washing fluid and autopsy material) by using real time hybridization-fluorescence detection of amplified products.

The results of PCR analysis are taken into account in complex diagnostics of disease.

2. PRINCIPLE OF PCR DETECTION

Detection of RNA of *Parainfluenza virus* types 1, 2, 3, and 4 includes RNA extraction from clinical material, reverse transcription of RNA into cDNA, and the real-time polymerase chain reaction (PCR). 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 linked to oligonucleotide probes, which bind specifically to the amplified product during thermocycling. The real-time monitoring of 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 (Internal Control STI-rec). It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition. **AmpliSens® *Parainfluenza virus*-FRT** PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using a wax layer. Wax melts and reaction components mix only at 95 °C.

3. CONTENT

AmpliSens® *Parainfluenza virus*-FRT PCR kit is produced in 2 forms:

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

AmpliSens® *Parainfluenza virus*-FRT PCR kit variant FRT (for use with iQ), **REF** R-V51(iQ,Dt)-CE;

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

Reagent	*Description*	*Volume, ml*	*Quantity*
PCR-mix-1-FL *hPiv* 1/3 (ready-to-use single-dose test tubes (*under wax)*)	colorless clear liquid	0.008	55 tubes of 0.2-ml volume
PCR-mix-1-FL *hPiv* 2/4 (ready-to-use single-dose test tubes (*under wax)*)	colorless clear liquid	0.008	55 tubes of 0.2-ml volume
PCR-mix-2-FL	colorless clear liquid	0.77	1 tube
Positive Control cDNA *hPiv* 1/3 (C+$_{hPiv\ 1/3}$)	colorless clear liquid	0.1	1 tube
Positive Control cDNA *hPiv* 2/4 (C+$_{hPiv\ 2/4}$)	colorless clear liquid	0.1	1 tube
Positive Control STI-88 (CS+)	colorless clear liquid	0.1	1 tube
TE-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C–)*	colorless clear liquid	1.2	1 tube
Internal Control STI-rec (IC)**	colorless clear liquid	0.12	5 tubes

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

** add 10 µl of Internal Control STI-rec during the RNA extraction procedure directly to the sample/lysis mixture.

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

4. ADDITIONAL REQUIREMENTS

- Transport medium (a reagent for storage of respiratory swabs)
- Reagent for pretreatment of sputum and aspirate of viscous consistency.
- Sterile saline solution or phosphate buffer for pretreatment of autopsy material.
- RNA/DNA extraction kit or automatic system for nucleic acid extraction.
- Reverse transcription kit.
- Disposable powder-free gloves and laboratory coat.
- Sterile RNase-free pipette tips with aerosol barriers (up to 200 µl and 1,000 µl) in racks.
- Tube racks.

- Vortex mixer.
- Desktop centrifuge.
- PCR box.
- Personal PCR cyclers (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia); Rotor-Gene Q (Qiagen, Germany); iCycler iQ, iQ5 (Bio-Rad, USA)).
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –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 mucous 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 DNA amplification techniques.

- The laboratory process must be one-directional, it should begin in the Extraction Area and then move 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 are 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 extraction kits from nasal and oropharyngeal swabs, sputum (or nasopharyngeal and tracheal aspirate), bronchoalveolar lavage or bronchial washes, and autopsy material (fragments of affected lungs).

Sampling

6.1 *Nasal swab* is taken with a sterile dry probe with a cotton swab. Before sampling, make the patient blow his nose if it is filled with mucus. Gently insert the probe through the nostril 2-3 cm deep towards the inferior nasal concha. Lower the probe and pass it under the inferior nasal concha. Rotate and remove the swab. Place the working part of the probe into a tube with 500 µl of Transport Medium for Storage and Transportation of Respiratory Swabs, **REF** 957-CE. Break off the probe and tightly secure the cap.

6.2 *Oropharyngeal swab* is taken with a sterile dry probe with a cotton swab. Before sampling make the patient rinse his mouth with water. Rotate the probe over the tonsillar area, palatine arches, and posterior area of the pharynx. Place the working part of the probe into the tube with Transport Medium for Storage and Transportation of Respiratory Swabs, REF 957-CE. Break off the probe and tightly secure the cap.

It is recommended to combine nasal and oropharyngeal 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.

6.3 *Sputum or nasopharyngeal or tracheal aspirate.* Sputum is collected to a sterile disposable container. Before sampling, make the patient rinse his mouth with water. *Nasopharyngeal or tracheal aspirate* is collected in accordance with the conventional technique and placed into a sterile disposable container.

6.4 *Bronchoalveolar lavage and bronchial washing fluid* are collected in accordance with the conventional technique and placed into a sterile disposable container.

Storage of the above-mentioned material before analysis is:

– at 2–8 °C for 1 day;

– at ≤ –16 °C for 1 week.

6.5 *Autopsy material* is to be placed to a sterile disposable container and frozen or analyzed within 1 hour. Material can be stored at ≤ –68 °C for 1 year. Only one freeze–thaw cycle is allowed.

Pretreatment

6.6 *Respiratory swabs*. Vortex and then centrifuge closed tubes at 5,000 rpm for 5 s to remove drops from the tube walls.

6.7 *Sputum or nasopharyngeal or tracheal aspirate.* Reagent Mucolysin, **REF** 180-CE manufactured by CRIE, is additionally required. Treat material according to the manufacturer's instructions. The prepared sample (100 µl) is used for RNA extraction. The remained sample can be frozen for further use.

6.8 *Bronchoalveolar lavage and bronchial washing fluid.* Use 100 µl of material for extraction. The remained sample can be frozen for further use.

6.9 *Autopsy material*. Homogenize the material with a sterile porcelain mortar and pestle. Then, prepare 10 % suspension in sterile saline or phosphate buffer. Transfer the suspension to a 1.5-ml tube, centrifuge at 10,000 rpm for 5 min, and use the supernatant (100 µl) for RNA extraction. The remained suspension can be frozen for further use.

7. WORKING CONDITIONS

AmpliSens® *Parainfluenza virus*-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1 RNA extraction

It is recommended to use the following nucleic acid extraction kits:

- RIBO-sorb, **REF** K2-1-Et-50-CE.
- RIBO-prep, **REF** K2-9-Et-50-CE.

NucliSENS easyMAG automated system can also be used.

8.1.1 RNA extraction with the use of RIBO-sorb reagent kit:

Extract RNA according to the manufacturer's instruction.

- Volume of the Internal Control STI-rec reagent added per each tube is10 µl;
- Volume of the Negative Control reagent added into the tube for Negative Control of Extraction (C–) is100 µl;
- Volume of clinical sample is 100 µl;
- If suspended particles are seen in the tubes after adding clinical samples (undissolved clinical material), centrifuge the tubes at 10,000 rpm for 1 min and transfer supernatant to new tubes

8.1.2 RNA extraction with the use of RIBO-prep reagent kit:

Extract RNA according to the manufacturer's instruction.

- Volume of the Internal Control STI-rec reagent added per each tube is10 µl;
- Volume of the Negative Control reagent added into the tube for Negative Control of Extraction (C–) is100 µl;
- Volume of clinical sample is 100 µl;
- Purified RNA can be stored at 2–8 °C for 4 hours, at ≤–16 °C for 1 month, at ≤–68 °C for a long time

8.1.3 RNA extraction with the use of NucliSENS easyMAG automated system

DNA extraction with NucliSENS easyMAG automated system is described in Guidelines [2].

8.2 Reverse transcription

It is recommended to use the following kit for complementary DNA (cDNA) synthesis on the RNA template:

- REVERTA-L (containing RT-G-mix-1), REF K3-4-50-CE or REF K3-4-100-CE.

Carry out the reverse transcription procedure according to the manufacturer's instruction.

8.3 Preparing real-time PCR

Amplification reaction should include the positive controls of amplification of the pathogens (see Table 1), positive control of amplification of the internal control CS+ (Positive Control STI-88), and the negative control of amplification, NCA, which is intended to check the purity of the reagents and accuracy of the technician. Moreover, the negative control of RNA extraction, C–, should be examined during amplification.

Table 1

Correspondence of the PCR-mixes-1-FL with Positive Controls of amplification

PCR-mix-1-FL	Positive Control samples
hPiv 1/3	Positive Control cDNA *hPiv* 1/3
hPiv 2/4	Positive Control cDNA *hPiv* 2/4

8.3.1 Preparing tubes for PCR

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

1. Take the required number of the tubes with the necessary **PCR-mix-1-FL** (see Table 1) and wax for amplification of cDNA from clinical and control samples. Make sure that wax covers the solution at the bottom completely.
2. Add **7 µl** of **PCR-mix-2-FL** to the surface of the wax layer of each tube ensuring that it does not fall under the wax and mix with **PCR-mix-1-FL**.
3. Add **10 µl** of **cDNA sample** obtained at the of reverse transcription stage per each tube.
4. Carry out the control reactions (**per each PCR-mix-1-FL, see Table 1**):

NCA - Add **10 µl** of **TE-buffer** to the tube labeled NCA (Negative Control of Amplification).
C+ - Add **10 µl** of the **required Positive Control** (see Table 1) to the tube labeled C+
CS+ - Add **10 µl** of **Positive Control STI-88** to the tube labeled CS+.
C– - Add 10 µl of the sample extracted from the Negative Control

5. Centrifuge the tubes for 1-2 s (if a plate-type instrument is used)

8.3.2 Amplification

1. Program the instrument according to manufacturer's manual, Important Product Information Bulletin, and Guidelines [2].
2. Create a temperature profile on your instrument as follows:

Table 2

Amplification program

	Rotor-type instruments[1]			**Plate-type instruments**[2]		
Step	Temperature, °C	Time	Cycle repeats	Temperature, °C	Time	Cycle repeats
1	**95**	**5 min**	1	**95**	**5 min**	1
2	**95**	10 s	10	**95**	10 s	10
	54	20 s		**54**	25 s	
	72	10 s		**72**	25 s	
3	**95**	10 s	35	**95**	10 s	35
	54	20 s Fluorescence signal detection		**54**	25 s Fluorescence signal detection	
	72	10 s		**72**	25 s	

Fluorescence is detected in the FAM/Green, JOE/Yellow/HEX, and ROX/Orange fluorescence channels.

3. Adjust the fluorescence channel sensitivity according to *Important Product Information Bulletin.*
4. Insert tubes into the reaction module of the device.
5. Run the amplification program with fluorescence detection.
6. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

Data analysis is performed by software of the used real-time PCR cycler. The results are interpreted by the crossing (or not-crossing) of the fluorescence curve with the threshold line in each channel that corresponds to the presence (or absence) of a *Ct* value of a cDNA sample in the corresponding column of the result grid.

Data analysis for each PCR-mix-1-FL should be carried out separately by selection of the tubes containing the required PCR-mix-1

Table 3

Correspondence of PCR-mixes-1-FL and detection channels

PCR-mix-1-FL	**Detection in the channel**		
	FAM/Green	**JOE/Yellow/HEX**	**ROX/Orange**
hPiv 1/3	Internal Control STI-rec	*hPiv* 3	*hPiv* 1
hPiv 2/4	Internal Control STI-rec	*hPiv* 2	*hPiv* 4

[1] For example, Rotor-Gene 3000, Rotor-Gene 6000 (Corbett Research, Australia), Rotor-Gene Q (Qiagen, Germany).
[2] For example, iCycler iQ, iQ5 (Bio-Rad, USA).

Interpretation of results

Principle of interpretation:

– RNA of the relevant type of *Parainfluenza virus* is **detected** in a sample if a *Ct* value is detected in the corresponding channel (see Table 3). Moreover, the fluorescence curve of this sample should cross the threshold line at the area of typical exponential growth of the fluorescence intensity.

– RNA of the relevant type of *Parainfluenza virus* is **not detected** in a sample if a *Ct* value is not detected (absent) in the corresponding channel (the fluorescence curve does not cross the threshold line), whereas the *Ct* value detected in the FAM/Green channel does not exceed the specified value.

– the result of analysis is **invalid** if the *Ct* value is not detected (absent) in the channel designated for detection of the pathogen (see Table 3) whereas the *Ct* value in FAM/Green channel is not detected or exceed the specified value. Repeat analysis of this sample starting with RNA extraction stage.

Boundary *Ct* values are specified in the Important Product Information Bulletin enclosed in the PCR kit

The results of analysis are considered reliable only if the results obtained for both Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Table 4).

Table 4

Results for controls

Control	**Stage for control**	***Ct* value in channel**		
		FAM/Green	**JOE/Yellow/HEX**	**ROX/Orange**
		Detection of Internal Control STI-rec	**Detection of pathogen**	**Detection of pathogen**
C–	RNA extraction	< boundary value*	No value	No value
NCA	PCR	No value	No value	No value
CS+	PCR	< boundary value*	No value	No value
C+	PCR	No value	< boundary value*	< boundary value*

* For boundary values, see the *Important Product Information Bulletin*.

10. TROUBLESHOOTING

The results of analysis are not taken into account in the following cases:

1. If no *Ct* value is detected in the Positive Control of amplification (C+) in the JOE/Yellow/HEX and ROX/Orange channel or if the *Ct* value detected in these

channels exceeds the boundary Ct value, amplification should be repeated for all negative clinical samples.

2. If a *Ct* value is detected in the channel intended for the pathogen detection in the Negative Control of extraction (C–) and/or Negative Control of amplification (NCA), analysis should be repeated (beginning with RNA extraction) for all samples that showed the presence of the pathogen RNA to rule out possible contamination.

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

11. TRANSPORTATION

AmpliSens® *Parainfluenza virus*-FRT PCR kit should be transported at 2–8 °C for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *Parainfluenza virus*-FRT** PCR kit are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® *Parainfluenza virus*-FRT** PCR kit are stable until the expiration date on the label. The shelf life of opened reagents is the same as that of unopened reagents, unless otherwise stated.

PCR-mix-1-FL *hPiv* 1/3 and PCR-mix-1-FL *hPiv* 2/4 are to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

Clinical material	Pathogen	DNA/RNA nucleic acid extraction kit	Reagent kit for amplification and detection	Analytical sensitivity, GE/ml [3]
Nasal and oropharyngeal swabs	*Parainfluenza virus* types 1-4	RIBO-sorb, RIBO-prep, NucliSENS easyMAG	PCR kit variant FRT	$1x10^3$

13.2. Specificity

The analytical specificity of **AmpliSens® *Parainfluenza virus*-FRT** PCR kit is ensured by selection of specific primers and probes as well as stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences deposited in gene banks by sequence comparison analysis

[3] Genome equivalent of a pathogen per 1 ml of a sample.

The PCR kit allows detection of cDNA fragments of *Parainfluenza virus* types 1, 2, 3, and 4 (*hPiv*).

The specific activity of the PCR kit was proved by analysis of clinical material with subsequent sequencing the amplified fragments of *Parainfluenza virus* types 1–4.

Nonspecific reactions were absent in tests with cDNA/DNA of the following viral pathogens: *Influenza virus* A and B, *Human Respiratory Syncytial virus* "Long" type A, *Human Rhinoviruses* (types 13, 15, 16, 17, 21, 26, and 29), *Coronavirus* causing TOPC (Frankfurt), *Coronavirus* causing feline infectious peritonitis (F1, F2, F5) and porcine gastroenteritis (*tr*ansmissible gastroenteritis *coronavirus*,TGC1, TGC8, TGC 9), *Herpes virus*, *Cytomegalovirus*, *Enteroviruses* (types Echo9, Echo30), as well as bacterial causative agents of acute respiratory disease (*Streptococcus* spp., *Staphylococcus aureus*, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Haemophilus influenzae, Moraxella catarrhalis, Legionella pneumophila*), normal microflora of human nasal cavity and oropharynx, and human cDNA/DNA. Nonspecific reactions were absent in tests of 60 cerebrospinal fluid (CSF) samples taken from patients with meningitis containing *Enterovirus* RNA and 100 samples of clinical material containing nucleic acids of *Respiratory Syncytial viruses* (types A and B), *Human Coronavirus* OC43, E229, NL63, HKUI, *Human Adenoviruses* groups B, C, and E, *Human Metapneumovirus* (types A and B), and *Human Bocavirus.*

The clinical specificity of **AmpliSens® *Parainfluenza virus*-FRT** PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES

1. Handbook "Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics", developed by Federal Budget Institute of Science "Central Research Institute for Epidemiology" of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being, Moscow, 2008.
2. Guidelines to the AmpliSens® *Parainfluenza virus*-FRT PCR kit for qualitative detection and differentiation of specific fragments of nucleic acids of *Parainfluenza virus* types 1 and 3 (genus *Respirovirus*) and *Parainfluenza virus* types 2 and 4 (genus *Rubulavirus*) in the clinical materials by using real-time hybridization-fluorescence detection developed by Federal Budget Institute of Science "Central Research Institute for Epidemiology".

15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science “Central Research Institute for 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.

16. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code	Σ	Sufficient for
IVD	*In vitro* diagnostic medical device		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation		Keep away from sunlight
	Manufacturer	**NCA**	Negative control of amplification
	Date of manufacture	**C–**	Negative control of extraction
EC REP	Authorised representative in the European Community	**CS+**	Positive Control STI-88
FBIS CRIE	Federal Budget Institute of Science “Central Research Institute for Epidemiology”	**C+**$_{hPiv\ 1/3}$ **C+**$_{hPiv\ 2/4}$	Positive Controls of Amplification

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
23.03.12 LA	Page footer	Reference number R-V51(iQ,Dt)-CE is added
	Throughout the text	A new format of the reagent kit was introduced
		Names of reagents were changed
		Intended use was given more correctly
		Real-time PCR instruments Rotor-Gene Q, iCycler iQ, iQ5 were added
		Brief description of extraction with RIBO-prep and reference to NucliSens easy MAG were added