CE

IVD

For Professional Use Only

AmpliSens® *hRSV*-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® *hRSV*-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Human Respiratory Syncytial virus* RNA in the clinical materials (nasal and oropharyngeal swabs, nasopharyngeal or tracheal sputum or aspirate, and autopsy material) by using real-time hybridization-fluorescence detection.

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

2. PRINCIPLE OF PCR DETECTION

Human Respiratory Syncytial virus detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special *Human Respiratory Syncytial virus* primers. In real-time PCR, the amplified product is detected using fluorescent dyes. These dyes are usually linked to oligonucleotide probes that bind specifically to the amplified product during thermocycling. The real-time 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® *hRSV*-FRT** PCR kit is a qualitative test that contains the Internal Control (IC). 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® *hRSV*-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® *hRSV*-FRT PCR kit is produced in 1 form:

AmpliSens® *hRSV*-FRT PCR kit variant FRT (for use with RG) **REF** R-V37(RG)-CE.

AmpliSens® *hRSV*-FRT PCR kit, variant FRT includes:

Reagent	***Description***	***Volume, ml***	***Amount***
PCR-mix-1-FEP/FRT *hRSV* ready-to-use single-dose test tubes (*under wax)*	colorless clear liquid	0.008	55 tubes of 0.2 ml
PCR-mix-2-FL	colorless clear liquid	0.77	1 tube
Positive Control cDNA *hRSV*-Flu (C+$_{hRSV\text{-}Flu}$)	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 (see RIBO-sorb, **REF** K2-1-Et-50-CE, RIBO-prep, **REF** K2-9-Et-50-CE protocols).

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

4. ADDITIONAL REQUIREMENTS

- RNA extraction kit.
- Reverse transcription kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2 ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia) or equivalent).
- Disposable polypropylene microtubes for PCR (0.2-ml; for example, Axygen, 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 and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, protect eyes while samples and reagents handling. Thoroughly wash hands afterward.
- 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 samples and unused reagents in compliance with local authorities requirements.
- Samples 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 another suitable disinfectant.
- Avoid contact with the skin, eyes, and mucosa. If skin, eyes, or mucosa contact, immediately flush with water and seek medical attention.
- 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® ***hRSV*-FRT** PCR kit is intended for the analysis of RNA extracted with RNA extraction kits from nasal and oropharyngeal swabs, nasopharyngeal or tracheal sputum or aspirate, and autopsy material.

Nasal swabs are taken using a probe with a dry cotton swab. Make the patient blow his nose if it is filled with mucus. Insert the probe gently along the external nasal wall by 2–3 cm till the inferior nasal concha. Then move the probe slightly lower, insert in the inferior nasal meatus under the inferior nasal concha, rotate, and pull it back along the external nasal wall.

When material is obtained, place the working area of the probe with a cotton swab into a sterile disposable tube with 500 µl of transport medium for storage and transportation of respiratory swabs. Break off the terminal part of the probe or cut it off to allow tight closing of the tube cup. Close the tube with the solution and the working area of the probe.

Oropharyngeal swabs are taken using a probe with a dry cotton swab. Obtain swabs by rotating the probe over the surface of tonsils, palatine arches, and the posterior wall of the pharynx after gargling of the oral cavity with water.

When material is obtained, place the working area of the probe with a cotton swab into a sterile disposable tube with 500 µl of transport medium for storage and transportation of respiratory swabs. Break off the terminal part of the probe or cut it off to allow tight closing of the tube cup. Close tube with the solution and the working area of the probe.

It is recommended to combine nasal and throat swabs. To do this, working parts of probes are placed into one tube with 500 µl of medium for storage and transportation of respiratory swabs and studied as one sample.

The sputum is collected into a sterile disposable container after preliminary gargling of the oral cavity with water. Nasopharyngeal or tracheal aspirates are taken by the conventional method and placed in a sterile disposable container.

Sectional material is placed in a sterile disposable container. Material is to be frozen after sampling or analyzed within 1 h.

Only one freeze–thaw cycle is allowed.

Pretreatment of material

Any operation with studied material is carried out in compliance with local authority's requirements.

All pretreatment manipulations are carried out using adjustable pipettes with disposable tips with aerosol barriers and disposable polypropylene tubes (1.5 ml or 10.0 ml). Disposable plastic dishes (tubes and tips) are to be discarded into a special container with a suitable disinfectant and utilized in compliance with local authority's requirements.

Swabs are used without preliminary preparation.

Sputum must be treated with Mucolysin reagent REF 180-CE according to the Mucolysin instruction manual. 100 µl of the pretreated sputum is used for RNA extraction.

Section material is homogenized with a sterile porcelain mortar and pestle, with subsequent preparation of a 10 % suspension in sterile saline or phosphate buffer. Transfer the suspension to 1.5-ml tube and centrifuge for 5 min at 10,000 rpm. 100 µl of the supernatant is used for RNA extraction. To repeat the test later, freeze the remained suspension at ≤ – 16 °C.

Additional reagents manufactured by Federal Budget Institute of Science "Central Research Institute for Epidemiology" are required:

1. Transport Medium for Storage and Transportation of Respiratory Swabs (unaliquoted) REF 959-CE is used for sampling and storage of nasal and oropharyngeal swabs.
2. Mucolysin reagent REF 180-CE is used for pretreatment of sputum and aspirates.

7. WORKING CONDITIONS

AmpliSens® *hRSV*-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.

Extract RNA according to the manufacturer's instructions.

Add **10 µl** of **Internal Control STI-rec** to each tube.

8.2. Reverse transcription

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

- REVERTA-L, **REF** K3-4-50-CE.

Carry out the reverse transcription according to the manufacturer's instructions.

8.3. Preparing PCR

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

8.3.1. Preparing tubes for PCR

1. Prepare the required number of the tubes with **PCR-mix-1-FEP/FRT *hRSV*** and wax for amplification of cDNA from clinical and control samples.
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-FEP/FRT *hRSV***.
3. Using tips with aerosol barrier, add **10 µl** of **cDNA** obtained in RNA reverse transcription reaction to the prepared tubes.
4. Carry out the control amplification reactions:

NCA - Add **10 µl** of **TE-buffer** to the tube labeled NCA (Negative Control of Amplification).

C+*hRSV*-Flu - Add **10 µl** of **Positive Control cDNA *hRSV*-Flu** to the tube labeled C+$_{hRSV\text{-}Flu}$ (Positive Control of Amplification).

CS+ - Add **10 µl** of **Positive Control STI-88** to the tube labeled CS+.

8.3.2. Amplification

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

Table 1

Programming thermocyclers at cDNA amplification of *Human Respiratory Syncytial Virus*

Step	*Temperature, °C*	*Time*	*Fluorescence detection*	*Cycle repeats*
Hold	95	5 min	–	1
Cycling 1	95	10 s	–	10
	54	20 s	–	
	72	10 s	–	
Cycling 2	95	10 s	–	35
	54	20 s	FAM/Green, JOE/Yellow	
	72	10 s	–	

3. Fluorescence is detected on the 2-nd step of stage Cycling 2 **(54 °C)** in FAM/Green and

JOE/Yellow fluorescence channels.

4. Adjust the fluorescence channel sensitivity according to Guidelines.

9. DATA ANALYSIS

IC is detected in the FAM/Green fluorescence channel, *Human Respiratory Syncytial Virus* RNA is detected in the JOE/Yellow fluorescence channel.

See **Guidelines** for data analysis settings for Rotor-Gene 3000 or Rotor-Gene 6000.

9.1. Interpretation of results

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

Table 2

Results for controls

Control	Stage for control	Ct value in channel		Interpretation
		FAM/Green	JOE/Yellow	
C–	DNA extraction	Pos (< boundary value*)	Neg	OK
NCA	Amplification	Neg	Neg	OK
C+*hRSV*-Flu	Amplification	Neg	Pos (< boundary value *)	OK
CS+	Amplification	Pos (< boundary value *)	Neg	OK

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

1. The sample is considered to be **positive** for *Respiratory Syncytial virus* if its Ct value does not exceed the boundary Ct value in the JOE/Yellow channel. If the Ct value exceeds the boundary Ct value in the JOE/Yellow channel, PCR should be repeated. If the same result is obtained or if the Ct value is less than the boundary Ct value, the sample is considered to be **positive**.
2. The sample is considered to be **negative** for *Respiratory Syncytial virus* if its Ct value is not detected in the results grid (the fluorescence curve does not cross the threshold line) in the JOE/Yellow channel and the Ct value in the results grid in the FAM/Green channel does not exceed the boundary Ct value.

10. TROUBLESHOOTING

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

1. If no signal is detected for the positive controls of amplification, it may suggest that the programming of the temperature profile of the used Instrument was incorrect, or that the configuration of the PCR reaction was incorrect, or that the storage conditions for kit components did not comply with the manufacturer's instruction, or that the reagent kit expired. Programming of the used instrument, storage conditions, and the expiration date of the reagents should be checked, and then PCR should be repeated.
2. If the Ct value is present for C– in the JOE/Yellow channel and for NCA in any channel in

the results grid, this indicates contamination of reagent or samples. In such cases, the results of analysis must be considered as invalid. Test analysis must be repeated and measures to detect and eliminate the source of contamination are to be taken.

3. If the Ct value in the FAM/Green channel (IC) exceeds the boundary Ct value, analysis of this sample should be repeated starting from the extraction stage.

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

11. TRANSPORTATION

AmpliSens® *hRSV*-FRT PCR kit should be transported at 2–8 ºC for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *hRSV*-FRT** PCR kit are to be stored at the temperature at 2–8 °C when not in use. All components of the **AmpliSens® *hRSV*-FRT** PCR kit are stable until the expiration date on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

PCR-mix-1-FEP/FRT *hRSV* is to be stored away from light.

13. SPECIFICATIONS

13.1. Sensitivity

Analytical sensitivity of **AmpliSens® *hRSV*-FRT** PCR kit is not less than $1x10^3$ genome equivalents per 1 ml of sample (GE/ml).

The claimed analytical performance characteristics of **AmpliSens® *hRSV*-FRT** PCR kit are guaranteed only when additional reagents kits RIBO-sorb, RIBO-prep and REVERTA-L (manufactured by Federal Budget Institute of Science “Central Research Institute for Epidemiology”) are used.

13.2. Specificity

The analytical specificity of **AmpliSens® *hRSV*-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. The clinical specificity of **AmpliSens® *hRSV*-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 State Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2008.

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®** ***hRSV*-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number		Sufficient for
LOT	Batch code		Expiration Date
IVD	*In vitro* diagnostic medical device		Consult instructions for use
VER	Version		Keep away from sunlight
	Temperature limitation	**NCA**	Negative control of amplification
	Manufacturer	**C–**	Negative control of extraction
	Date of manufacture	**C+*hRSV*-Flu, CS+**	Positive control of amplification
EC REP	Authorised representative in the European Community	**IC**	Internal control
	Caution		

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
13.12.10	Cover page	The phrase “For Professional Use Only” was added
	Intended use	The phrase “The results of PCR analysis are taken into account in complex diagnostics of disease” was added
	Content	New sections “Working Conditions” and “Transportation” were added
		The “Explanation of Symbols” section was renamed to “Key to Symbols Used”
	Stability and Storage	The information about the shelf life of reagents before and after the first use was added
		Information that PCR-mix-1-FEP/FRT *hRSV* is to be kept away from light was added
	Key to Symbols Used	The explanation of symbols was corrected
	Text	Positive Control STI was changed to Positive Control STI-88
		Positive Control cDNA *hRSV*-Flu (C+) was changed to Positive Control cDNA *hRSV*-Flu (C+$_{hRSV\text{-Flu}}$)
22.06.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”