



IVD For *In Vitro* Diagnostic Use

AmpliSens® hRSV-FRT

PCR kit

Instruction Manual

AmpliSens®

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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, throat swabs; sputum or aspirate of nasopharynx or trachea; autopsy material) by using real-time hybridization-fluorescence detection.

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 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® hRSV-FRT PCR kit is a qualitative test, which contain 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® hRSV-FRT PCR kit uses "hot-start", which greatly reduces frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by separation of nucleotides and Taq-polymerase by using wax layer. Wax melting and reaction mix components occur 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 (<i>under wax</i>)	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+)	colorless, clear liquid	0.1	1 tube
Positive Control STI (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**	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, "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 isolation 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 with 0.5 ml (0.2) capacity.
- Refrigerator for temperature between 2 and 8 °C.
- Deep-freezer with temperature not more than minus16°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 other suitable disinfectant.
- Avoid contact with the skin, eyes and mucosa. If skin, eyes and mucosa contact immediately flush with water, seek medical attention.
- 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.
- The laboratory process must be one directional, it should begin in the Extraction Area 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 is 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 by RNA isolation kits from nasal, throat swabs, sputum or aspirate of nasopharynx or trachea, autopsy material.

Nasal swabs are obtained by probe with dry cotton swab. Blow one's nose if it is filled by mucus. Insert probe gently along the external nasal wall on 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 remove along the external nasal wall.

When material is obtained insert the working area of the probe with cotton swab to sterile disposable tube with 500 µl of transport medium for storage and transportation of respiratory swabs. Broke off the terminal part of the probe or cut it off to allow dense closing of tube cap. Close tube with solution and working area of the probe.

Throat swabs are obtained by probe with dry cotton swab. Obtain smears by rotating the probe at the surface of tonsils, palatine arches, posterior wall of pharynx after gargling of oral cavity with water.

When material is obtained insert the working area of the probe with cotton swab to sterile disposable tube with 500 µl of transport medium for storage and transportation of respiratory swabs. Broke off the terminal part of the probe or cut it off to allow dense closing of tube cap. Close tube with solution and working area of the probe.



It is recommended to combine nasal and throat swabs. For this working ends of probes are placed in one tube with 500 µl of medium for storage and transportation of respiratory swabs and studied as one sample.

The **sputum** is collected in a sterile disposable container after preliminary gargling of oral cavity with water. **Aspirates of nasopharynx** or **trachea** are obtained by traditional method and placed in a sterile disposable container.

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



Only one freeze-thaw cycle is allowed.

Material's preliminary preparation.

Any operation with studied material transportation is carried out in compliance with local authorities requirements.

All manipulations, connected with probes preparation, are carried out by varying volume pipettors with using of disposable polypropylene microtubes of 1.5 ml or 10.0 ml volume and tips with aerosol barriers. Disposable plastic dishes (tubes, tips) are to be thrown into the special container with suitable disinfectant. They are to be utilized in compliance with local authorities requirements.

Swabs are used without preliminary preparation.

Sputum must be treated with "Mucolysin" reagent [REF] 180, according to "Mucolysin" manual. 100 µl of pretreated sputum is used for RNA extraction.

Section material is homogenized with sterile porcelain mortar and pestle, with subsequent preparation of 10 % suspension on sterile saline or phosphate buffer. Transfer suspension to 1.5 ml tube and centrifuge 5 min at 10000 rpm. 100 µl of pretreated supernatant is used for RNA extraction. In case of test repeat freeze the rest of the suspension at no more than minus 16 °C.

Additional reagents (manufactured by Federal State Institution of Science Central Research Institute of Epidemiology) are required:

1. "Transport medium for storage and transportation of respiratory swabs" [REF] 959 is used for sampling and storage of nasal and throat swabs.
2. "Mucolysin" reagent [REF] 180 is used for preliminary treatment of sputum and aspirates.

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.
- "RIBO-prep", [REF] K2-9-Et-50-CE.



Carry the RNA isolation according to the manufacturer's instructions.



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

7.2. Reverse transcription.

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

- "REVERTA-L", [REF] K3-4-50-CE.



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

7.3. Preparing the PCR.

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

7.3.1 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, into prepared tubes.
4. Carry the control amplification reactions:

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

C+ Add 10 µl of **Positive Control cDNA hRSV-Flu** to the tube labeled C+ (Positive Control of Amplification).

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

7.3.2. Amplification.

1. Program the Rotor-Gene™ according to manufacturer's manual and Appendix 1.
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 sec	–	10
	54	20 sec	–	
	72	10 sec	–	
Cycling 2	95	10 sec	–	35
	54	20 sec	FAM/Green, JOE/Yellow	
	72	10 sec	–	

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

8. DATA ANALYSIS.

Internal Control is detected on the FAM/Green fluorescence channel, *Human Respiratory Syncytial virus* RNA is detected on the JOE/Yellow fluorescence channel.

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

8.1. Results interpretation.

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

Table 1

Results for controls

Control	Stage for control	Ct value on channel		Interpretation
		FAM/Green	JOE/Yellow	
C-	DNA isolation	Pos (< X*)	Neg	OK
NCA	Amplification	Neg	Neg	OK
C+	Amplification	Neg	Pos (< Y*)	OK
CS+	Amplification	Pos (< X*)	Neg	OK

*For X, Y values see Appendix 1.

- The sample is considered to be positive for *Respiratory Syncytial virus* if its Ct value does not exceed Y on JOE/Yellow channel. If Ct value exceed Y on JOE/Yellow channel, the PCR should be repeated. In case of result repeating or if Ct value less than Y is obtained, the sample is considered to be positive.
- The sample is considered to be negative for *Respiratory Syncytial virus* if its Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) on JOE/Yellow channel and in the results grid on the FAM/Green channel the Ct value doesn't exceed X.

9. TROUBLESHOOTING.

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

- If no signal is detected for Positive Controls of amplification, it can suggest incorrect programming of the temperature profile of used Instrument, incorrect configuration of the PCR reaction or storage conditions for kit components has not complied with manufacturer instruction, or the reagents kit has expired. Programming of the used Instrument, storage conditions, and the expiration date of the reagents should be checked, and then the PCR should be repeated.
- If the Ct value is present for the Negative Control of extraction (C-) on JOE/Yellow channel and for Negative Control of Amplification on any channels in the results grid, it indicates the contamination of reagent or samples. In such cases results of analysis must be considered as irrelevant. Test analysis must be repeated and measures to detect and eliminate the source of contamination are to be taken.
- If Ct value on FAM/Green channel (IC) exceed X, analysis of this sample should be repeated from extraction stage.

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

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens® hRSV-FRT** PCR kit is not less than 1×10^3 genome equivalents per 1 ml of sample (GE/ml).



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

11.2. Specificity.

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. Specificity of **AmpliSens® hRSV-FRT** PCR kit was confirmed in laboratory clinical trials.

12. REFERENCES.

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

14. EXPLANATION OF SYMBOLS.



Manufacturer



Use by



For *in Vitro* Diagnostic Use



Catalogue number



Contains sufficient for <n> tests



Consult instructions for use



For working with Rotor-Gene™ 3000/6000



Positive control



Temperature limitation



Batch code



Version



Internal Control complex



Authorized representative in the European Community.



Caution, consult accompanying documents



For working with iQ5, iQ iCycler



Negative control