



For *in Vitro* Diagnostic Use

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AmpliSens[®] hRSV-EPh PCR kit

Instruction Manual

AmpliSens[®]



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1. INTENDED USE.

AmpliSens® hRSV-EPh PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *human respiratory syncytial virus* RNA in the clinical material (nasopharyngeal swabs, throat swabs, sputum) by using electrophoretic detection of the amplified products in agarose gel.

2. PRINCIPLE OF PCR DETECTION.

Human respiratory syncytial virus detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen cDNA specific region using special *hRSV* primers. After PCR the amplified product is detected in agarose gel. **AmpliSens® hRSV-EPh** 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-EPh** 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-EPh PCR kit is produced in 2 forms:

AmpliSens® *hRSV-EPh* PCR kit variant 50 R (tubes 0.5 ml), **REF** V37-50-R0,5-CE.

AmpliSens® *hRSV-EPh* PCR kit variant 50 R (tubes 0.2 ml), **REF** V37-50-R0,2-CE.

AmpliSens® hRSV-EPh PCR kit variant 50 R includes:

Reagent	Description	variant 50 R	
		Volume (ml)	Quantity
PCR-mix-1-R hRSV ready-to-use single-dose test tubes (under wax)	colorless, clear liquid	0.005	55 tubes of 0.5 or 0.2 ml
PCR-mix-2 red	clear red liquid	0.6	1 tube
Mineral oil for PCR	colorless viscous liquid	2.0	1 dropper bottle
Positive Control cDNA hRSV (C+)	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-550-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 during the RNA isolation procedure directly to the sample/lysis mixture

(see “RIBO-sorb”, **REF** K2-1-Et-50-CE protocol).

AmpliSens® *hRSV-EPh* PCR kit variant 50 R is intended for 55 reactions, including controls.

4. ADDITIONAL REQUIREMENTS

- RNA isolation kit.
- Reverse transcription kit.
- Agarose gel detection kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200 µl).
- Vortex mixer.
- PCR box.
- Thermostatic bath or dry block for tubes with controlled temperature and capability to incubate at temperature between 25 °C and 100 °C.
- Tube racks.
- Personal thermocyclers (for example, “Gradient Palm Cycler” (“Corbett Research”, Australia), “MAXYGENE”, (“Axygen”, USA) or equivalent);
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity (for example, “Axygen”, USA).
- Refrigerator with 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 to read this handbook before starting work



Mucolysin **REF** R180 and Transport medium for swabs **REF** R959 reagents are additionally needed for sampling. This kit does not contain them.

AmpliSens[®] hRSV-EPh PCR kit is intended for analysis of RNA extraction by RNA isolation kits from:

- *Nasopharyngeal mucous membrane swabs without preliminary preparation*
- *Throat mucous membrane swabs without preliminary preparation*
- *Sputum (in disposable containers) after preliminary preparation*

Nasopharyngeal mucous membrane swabs are obtained by probe with dry cotton tampon. It is to be gently inserted into the nostril along the superior nose wall to the deep of 2-3 cm up to the inferior nasal concha. The tampon is to be gone down slightly, inserted into inferior nasal meatus under inferior nasal concha. Then the circular movement is to be done and the tampon is to be removed from the nose along superior nose wall. After sampling the tampon (working part of the probe with cotton tampon) is to be placed into the disposable tube with 500 µl of transport medium (sterile saline or phosphate buffer solutions also can be used). Then the probe is to be broken and the tube is to be tightly closed.

Throat mucous membrane swabs are obtained by probe with dry cotton tampon from tonsillar area, palatine arches, and posterior oropharyngeal surface with help of circular movements.

After sampling the tampon (working part of the probe with cotton tampon) is to be placed into the disposable tube with 500 µl of transport medium (sterile saline or phosphate buffer solutions also can be used). Then the probe is to be broken and the tube is to be tightly closed.



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.



Only one freeze-thaw cycle of clinical material is allowed.

7. PROTOCOL

7.1. RNA Isolation

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

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



Carry the RNA isolation in compliance with the manufacturer protocol.

7.2. Reverse transcription

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

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



Carry out the reverse transcription in compliance with the manufacturer protocol.



cDNA, obtained by reverse transcription, is to be 3 times diluted by DNA buffer. It's should be done as follows: 40 µl of DNA buffer is to be added to 20 µl of cDNA by individual tip.

7.3. Preparing the PCR.

Total reaction volume - 25 µl, volume of cDNA sample - 10 µl.

7.3.1 Preparing tubes for PCR.

1. Prepare the required number of PCR tubes with **PCR-mix-1-R hRSV** and wax for amplification of cDNA from clinical and control samples.
2. Add **10 µl of PCR-mix-2 red** to the surface of wax layer, of each tube ensuring that it does not fall under the wax and mix with PCR-mix-1-R *hRSV*.
3. Add above 1 drop of **mineral oil for PCR** (about 25 µl).

7.3.2 Amplification.

Use prepared tubes for PCR. Under or directly on the level of oil, using tips with aerosol barrier add **10 µl of cDNA samples**, obtained from clinical or control samples at the stage of reverse transcription.

Carry out the **control amplification reactions**:

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

C+ -Add 10 µl of **Positive Control cDNA hRSV** to the tube for Positive Control of Amplification.

Run the following program on the thermocycler (see table 1). When the temperature reaches 95°C (pause regimen), insert tubes to cells of amplifier and press button to continue.

It is recommended to precipitate drops from walls of tubes by short vortex (1–3 sec) before their insertion in thermocycler.

Programming thermocyclers at cDNA amplification of *human respiratory syncytial virus*

step	Thermocyclers with active temperature adjustment:						Thermocyclers with block temperature adjustment:		
	"GeneAmp PCR System 2400" (Applied Biosystems); "Terzik" (DNA-Technology)			"GeneAmp PCR System 2700" (Applied Biosystems); "Gradient Palm Cycler" (Corbett Research); «MAXYGENE», (Axygen)			"Uno-2" (Biometra), "MiniCycler", "PTC-100" (MJ Research)		
	temperature	time	cycles	temperature	time	cycles	temperature	time	cycles
0	95 °C	pause		95 °C	pause		95 °C	pause	
1	95 °C	5 min	1	95 °C	5 min	1	95 °C	5 min	1
2	95 °C	10 sec	42	95 °C	10 sec	42	95 °C	45 sec	42
	56 °C	10 sec		56 °C	25 sec		56 °C	45 sec	
	72 °C	10 sec		72 °C	25 sec		72 °C	45 sec	
3	72 °C	1 min	1	72 °C	1 min	1	72 °C	1 min	1
4	10 °C	storage		4 °C	storage		10 °C	storage	

Amplification in thermocycler with block temperature adjustment lasts 2 h, in thermocycler with active temperature adjustment — 1 h 30 min.

After the reaction is finished PCR tubes must be collected and sent to the room for PCR products analysis.

Analysis of amplification products is performed by separation of DNA fragments in agarose gel.

The amplified samples can be stored for 16 h at room temperature, for 1 week at 2 – 8 °C (be sure of samples heating to the room temperature before running electrophoresis).

8. DATA ANALYSIS.

It's recommended to use the following detection agarose kit:

- "EPh" variant 200, **REF** K5-200-CE.

Analysis of results is based on the presence or absence of specific bands of amplified cDNA in agarose gel (1.7%). The length of specific amplified cDNA fragments is:

- *hRSV* - 298 bp
- IC STI-550-rec - 550 bp



Put the protective mask or use the glass filter while watching and photographing the gel

Results interpretation

Table 2

Results for controls

Control	Controlled step	Specific bands in the agarose gel		Interpretation
		298 bp	550 bp	
C-	RNA isolation	No	Yes	OK
NCA	Amplification	No	No	OK
C+	Amplification	Yes	No	OK

- The sample is considered to be positive for *human respiratory syncytial virus* RNA if the band of 298 bp is present in agarose gel. The band of IC (550 bp) could be absent in the samples with high concentration of *hRSV* RNA.
- The sample is considered to be negative for *human respiratory syncytial virus* RNA if the band of 298 bp is absent and the band of 550 bp is present.

Besides specific bands the indistinct washed-out bands of primer-dimers may be seen in lanes, they are situated lower than level of 100 bp of nucleotide pairs.

9. TROUBLESHOOTING.

Analysis results are not obtained as per the following examples:

- If results of control samples analysis do not correspond to the listed above (Table 2), then the tests are to be repeated.
- If in lanes none of bands of 298 and 550 nucleotide pairs is observed, result of analysis for this sample is irrelevant and the analysis of this sample should be repeated from the very beginning. It can be caused by mistake in clinical material processing that provoked loss of RNA/DNA or inhibition of RT and/or PCR.
- If in lanes nonspecific bands at different levels are presented, it may be caused by lack of “hot start” or false temperature regimen in thermocycler.
- If in lanes corresponding to negative control (NCA, C-) specific band of 298 bp appears it means that reagents or samples contamination has taken place. In such cases results of analysis must be considered as irrelevant. Test analysis must be repeated and measures for detecting contamination source must be undertaken.

10. STABILITY AND STORAGE.

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

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of AmpliSens® *hRSV*-EPh PCR kit is no less than 5×10^3 genome equivalents per 1 ml of a sample (GE/ml).



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

11.2. Specificity.

Specificity of AmpliSens® *hRSV*-EPh PCR kit is ensured by selection of specific primers and strict reaction conditions as well as laboratory and clinical trials.

12. REFERENCES.

1. Manual “Sampling, transportation and 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 accordance with Federal State Institution of Science “Central Research Institute of Epidemiology” ISO 13485 – certified Total Quality Management System, each lot of AmpliSens® *hRSV*-EPh PCR kit is 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