



For *in Vitro* Diagnostic Use

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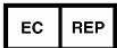
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# AmpliSens<sup>®</sup> *Influenza virus A/B-EPh*

## PCR kit

### Instruction Manual

# AmpliSens<sup>®</sup>



Ecoli s.r.o., Studenohorská 12  
 841 03 Bratislava 47  
 Slovak Republic  
 Tel.: +421 2 6478 9336  
 Fax: +421 2 6478 9040  
[ecoli@ecoli.sk](mailto:ecoli@ecoli.sk)  
[www.ecoli.sk](http://www.ecoli.sk) [www.pcrdiagnostics.eu](http://www.pcrdiagnostics.eu)



Federal State Institution of Science  
 Central Research Institute of Epidemiology  
 3A Novogireevskaya Street  
 Moscow 111123 Russia

## 1. INTENDED USE.

**AmpliSens® Influenza virus A/B-EPh** PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Influenza virus A* and *Influenza virus B* RNA in the clinical material (nasal, throat swabs; sputum or aspirate of nasopharynx or trachea; autopsy material) by using electrophoretic detection of the amplified products in agarose gel.

## 2. PRINCIPLE OF PCR DETECTION.

*Influenza virus A* and *Influenza virus B* detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen cDNA specific region using special *Influenza virus A* and *B* primers. **AmpliSens® Influenza virus A/B-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® Influenza virus A/B-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. The wax melting and reaction mix component occurs only at 95°C.

## 3. CONTENT.

**AmpliSens® Influenza virus A/B-EPh** PCR kit is produced in 2 forms:

**AmpliSens® Influenza virus A/B-EPh** variant 50 R (vials 0.5 ml) **REF** V36-50-R0,5;

**AmpliSens® Influenza virus A/B-EPh** variant 50 R (vials 0.2 ml) **REF** V36-50-R0,2.

**AmpliSens® Influenza virus A/B-EPh** variant 50 R includes:

Reagent	Description	Volume (ml)	Quantity
<b>PCR-mix-1-R Influenza virus A-IC</b> ready-to-use single-dose test tubes ( <i>under wax</i> )	colorless, clear liquid	0.005	55 tubes of 0.5 or 0.2 ml
<b>PCR-mix-1-R Influenza virus B</b> ready-to-use single-dose test tubes ( <i>under wax</i> )	colorless, clear liquid	0.005	55 tubes of 0.5 or 0.2 ml
<b>PCR-mix-2 red</b>	red, clear liquid	1.2	1 tube
<b>Mineral oil for PCR</b>	colorless, viscous liquid	4.0	1 vial
<b>Positive Control cDNA Influenza virus A (C<sub>A</sub>+)</b>	colorless, clear liquid	0.1	1 tube
<b>Positive Control cDNA Influenza virus B (C<sub>B</sub>+)</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-550-rec (IC)**</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 during the RNA isolation procedure directly to the sample/lysis mixture (see

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

**AmpliSens® Influenza virus A/B-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 RNase-free pipette tips with aerosol barriers (up to 200 µl).
- Vortex mixer.
- Desktop microcentrifuge with rotor for 2 ml reaction tubes (RCF max. 16,000 x g)
- PCR box.
- Vacuum aspirator with flask for removing supernatant.
- Thermostatic bath or dry block for tubes with controlled temperature and capability to incubate at temperature from 25°C to 100 °C.
- Tube racks.
- Personal thermocyclers (for example, Terzik (DNA-Technology, Russia), Gradient Palm Cycler (Corbett Research, Australia), Maxygene (Axygen Scientific, USA)).
- Refrigerator with temperature from 2 to 8 °C.
- Deep-freezer with temperature not more than minus 16 °C.
- Waste bin for used tips.
- Permanent pen for labeling.

## 5. GENERAL PRECAUTIONS.

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and put the new tip for every procedure.
- Store and handle amplicons separately from all other reagents.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Wear protective gloves, laboratory cloths, 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 biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills with 0.5% sodium hypochlorite solutions or other suitable disinfectant.
- Avoid contact with the skin, eyes and mucosa membranes. If skin, eyes and mucosa membranes contact immediately flush with water, seek medical attention.
- Material Safety Data Sheets (MSDS) are available on request.
- The product usage should be allowed only for personnel trained to the DNA amplification techniques.
- The laboratory process must be unidirectional; it should begin in the Extraction Area move to the Amplification and Detection Area. Do not return samples, equipment and reagents to the area where you carried out the previous step.



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

## 6. SAMPLING AND HANDLING.



Obtaining of biological materials samples 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® Influenza virus A/B-Eph** PCR kit is intended for analysis of RNA extracted with RNA isolation kits from in the clinical material (nasal and throat swabs; sputum; autopsy material).



For trachea sputum and aspirate pretreatment please use "Mucolysin" reagent **REF** R13.

## 7. PROTOCOL.

### 7.1. RNA Isolation.

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

- "RIBO-sorb", **REF** K2-1-Et-50;



Carry out the RNA isolation in compliance with the manufacturer protocol.  
The volume of clinical sample is 100 µl.  
The volume of Internal Control STI-rec (IC) is 10 µl.

### 7.2. Reverse transcription

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

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



Carry out the reverse transcription in compliance with the manufacturer protocol.  
Dilute the cDNA sample **3-fold** with RNA-buffer: add 40 µl of RNA-buffer to 20 µl of the sample and mix carefully.

### 7.3. Preparing the PCR.

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

#### 7.3.1 Detection of *Influenza virus A* RNA.

1. Prepare the required number of tubes with **PCR-mix-1-R *Influenza virus A*-IC** and wax for amplification of cDNA from clinical and control samples.
2. Add **10 µl** of **PCR-mix-2 red** 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-R *Influenza virus A*-IC**.
3. Add above **1 drop** of **mineral oil for PCR** (about **25 µl**).
4. Using tips with aerosol barrier add **10 µl cDNA samples** obtained from clinical or control samples.
5. Carry out the control amplification reactions:

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

C+A -Add **10 µl** of **Positive Control cDNA *Influenza virus A*** to the tube labeled C+A.

#### 7.3.2 Amplification (*Influenza virus A*).

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

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

Table 1

Programming thermocyclers for *Influenza virus A* cDNA amplification

Step	Thermocyclers with active temperature adjustment:						Thermocyclers with block temperature adjustment:		
	"Terzik" (DNA-Technology), "GeneAmp PCR System 2400" (Applied Biosystems)			"GeneAmp PCR System 2700" (Applied Biosystems), "Gradient Palm Cycler" (Corbett Research), «Maxygene» (Axygen Scientific)			"Biometra", "MiniCycler", "PTC-100" (MJ Research)		
Step	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	1 min	42
	63 °C	10 sec		63 °C	25 sec		63 °C	1 min	
	72 °C	10 sec		72 °C	25 sec		72 °C	1 min	
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	

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 to warm the samples to room temperature before running electrophoresis).

#### 7.3.3 Detection of *Influenza virus B* RNA.

1. Prepare the required number of tubes with **PCR-mix-1-R *Influenza virus B*** and wax for amplification of cDNA from clinical and control samples..
2. Add **10 µl** of **PCR-mix-2 red** 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-R *Influenza virus B***.
3. Add above **1 drop** of **mineral oil for PCR** (about **25 µl**).
4. Using tips with aerosol barrier add **10 µl cDNA samples** obtained from clinical or control samples.
5. Carry out the control amplification reactions:

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

C+B -Add **10 µl** of **Positive Control cDNA *Influenza virus B*** to the tube labeled C+B.

### 7.3.2 Amplification (*Influenza virus B*).

Run the following program on the thermocycler (see Table 2). When the temperature reaches 95°C (pause regimen), insert tubes into the cells of amplifier and press the button to continue.

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

Table 2

Programming thermocyclers for *Influenza virus B* cDNA amplification

Step	Thermocyclers with active temperature adjustment:						Thermocyclers with block temperature adjustment:		
	"Terzik" (DNA-Technology), "GeneAmp PCR System 2400" (Applied Biosystems)			"GeneAmp PCR System 2700" (Applied Biosystems), "Gradient Palm Cycler" (Corbett Research), «Maxygene» (Axygen Scientific)			"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	1 min	42
	63 °C	10 sec		63 °C	25 sec		63 °C	1 min	
	72 °C	10 sec		72 °C	25 sec		72 °C	1 min	
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	

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 to warm the samples to room temperature before running electrophoresis).

### 8. DATA ANALYSIS.

It's recommended using of the following detection agarose kit:

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

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

- *Influenza virus A* - 365 bp
- *Influenza virus B* - 196 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 3

Results for controls

Control	Controlled step	Specific bands in the agarose gel			Interpretation
		365 bp	196 bp	550 bp	
C-	RNA isolation	No	NA*	Yes	OK
NCA	Amplification	No	No	No	OK
C+A	Amplification	Yes	NA*	No	OK
C+B	Amplification	NA*	Yes	No	OK

\* Note, that the C and C+A are **not analyzed (NA)** on PCR-mix-1-R *Influenza virus B* and C+B is *not analyzed* on PCR-mix-1-R *Influenza virus A*.

- The sample is considered to be positive for *Influenza virus A* RNA if the band of 365 bp is present in agarose gel. The band of IC (550 bp) could be absent in the samples with high concentration of *Influenza virus A* RNA.
- The sample is considered to be positive for *Influenza virus B* RNA if the band of 196 bp is present in agarose gel.
- The sample is considered to be negative for *Influenza virus A* RNA if the band of 365 bp is absent and the band of 550 bp is present.
- The sample is considered to be negative for *Influenza virus B* RNA if the band of 196 bp is absent and the 550 bp band or 365 bp band were present at the test for *Influenza virus A*.

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 the results of control samples do not correspond to the listed above (Table 3), then the tests should be repeated.
- For *Influenza virus A* detection: if in lanes none of bands of 365 and 550 nucleotide pairs were observed for analyzed clinical sample, the test result of this sample is irrelevant and the analysis should be repeated from the very beginning. It can be caused by mistake in clinical processing that provoked loss of RNA/DNA or inhibition of RT and/or PCR.
- If in lines nonspecific bands are presented at different levels, 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 365 and/or 196 bp appears, it means that reagents or samples contamination has taken place. In such cases analysis results must be considered as irrelevant. Test analysis should be repeated and measures for detecting contamination source must be undertaken.

**10. STABILITY AND STORAGE.**

All components of **AmpliSens® Influenza virus A/B-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® Influenza virus A/B-EPh** PCR kit is no less than  $1 \times 10^3$  copies per 1 ml of a sample (cop/ml).



The claimed analytical features of **AmpliSens® Influenza virus A/B-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® Influenza virus A/B-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 compliance with Federal State Institution of Science Central Research Institute of Epidemiology ISO 13485 – certified Total Quality Management System, each lot of **AmpliSens® Influenza virus A/B-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
	Contains sufficient for <n> tests		Authorised representative in the European Community.
	Consult instructions for use		Caution, consult accompanying documents
	Positive Control		Negative control