



IVD

For *in Vitro* Diagnostic Use

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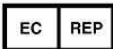
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AmpliSens[®] Influenza virus A/H1-swine-FEP

PCR kit

Instruction Manual

AmpliSens[®]



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1. INTENDED USE.

AmpliSens® Influenza virus A/H1-swine-FEP PCR kit is an *in vitro* nucleic acid amplification test for identification of *Influenza virus A/H1-swine* cDNA by using end point hybridization-fluorescence detection of amplified products.

2. PRINCIPLE OF PCR DETECTION.

Influenza virus A/H1-swine detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special *Influenza virus A/H1-swine* primers. In end point PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product in ordinary thermocycler. A multi channel rotor-type fluorometer is specially designed to detect fluorescent excitation from the fluorophores in a reaction mix after PCR. It allows the accumulating product detection without re-opening the reaction tubes after the PCR run. **AmpliSens® Influenza virus A/H1-swine-FEP** PCR kit is a qualitative test which contains 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/H1-swine-FEP** 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. The wax melting and reaction mix component occurs only at 95 °C.

3. CONTENT.

AmpliSens® Influenza virus A/H1-swine-FEP PCR kit is produced in 2 forms:

AmpliSens® Influenza virus A/H1-swine-FEP PCR kit (0,5 ml tubes),

REF V55-50-R0,5-FEP-CE;

AmpliSens® Influenza virus A/H1-swine-FEP PCR kit (0,2 ml tubes),

REF V55-50-R0,2-FEP-CE.

AmpliSens® Influenza virus A/H1-swine-FEP PCR kit includes:

Reagent	Description	Volume (ml)	Amount
PCR-mix-1-FEP/FRT <i>Influenza virus A/H1-swine</i> ready-to-use single-dose test tubes (<i>under wax</i>)	colorless, clear liquid	0.008	55 tubes of 0.5 or 0.2 ml
PCR-mix-2-FL	colorless, clear liquid	0.77	1 tube
PCR-mix-Background	colorless, clear liquid	0.5	1 tube
Mineral oil for PCR	colorless viscous liquid	4.0	1 dropper bottle

Positive Control cDNA <i>Influenza virus A/H1-swine</i> (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 (IC)**	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, RIBO-prep **REF** K2-9-Et-100-CE protocols).

AmpliSens® Influenza virus A/H1-swine-FEP PCR kit is intended for 55 reactions, including controls.

4. ADDITIONAL REQUIREMENTS.

- RNA isolation kit.
- Reverse transcription kit.
- Disposable powder-free gloves.
- Pipettes (adjustable).
- Sterile RNase-free pipette tips with aerosol filters (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2 ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Gradient Palm Cycler (Corbett Research, Australia or equivalent instrument).
- Fluorometer ALA-1/4 (“Biosan”, Latvia) or equivalent instrument.
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity (for example, “Axygen”, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer with temperature not more than –16 °C.
- Waste bin for used tips.

5. GENERAL PRECAUTIONS.

The user should always pay attention to the following:

- Use sterile RNase-free pipette tips with aerosol filters 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, protect eyes while samples and reagents handling. 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 samples and unused reagents in compliance with local authorities requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in

compliance 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 mucose membranes. If skin, eyes and mucose membranes 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® *Influenza virus A/H1-swine*-FEP PCR kit is intended for analysis of cDNA samples, in which the RNA of *Influenza virus A* was detected during analysis of clinical material or virus cultures with following kits of reagents:

- AmpliSens® *Influenza virus A/B*-FL;
- AmpliSens® *Influenza virus A/H5N1*-FL.

7. PROTOCOL.

7.1. RNA Isolation.

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

- "RIBO-sorb", **REF** K2-1-Et-50-CE.
- "RIBO-prep", **REF** K2-9-Et-100-CE.



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

7.2. Reverse transcription.

It's recommended to use the following reverse transcription reagents kits for complementary DNA (cDNA) synthesis from RNA.

- REVERTA-L, form 1 **REF** K3-4-50-CE, which contains RT-G-mix-1.
- REVERTA-L, form 2 **REF** K3-4-100-CE, which contains RT-G-mix-1.



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

7.3. Preparing the PCR.

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

7.3.1. Preparing tubes for PCR.

1. Prepare the required number of tubes with **PCR-mix-1-FEP/FRT *Influenza virus A/H1-swine*** and wax for amplification of cDNA from clinical and control samples.



Choice of amplification tubes depends on the instruments used:

- 0,2 ml tubes are used for thermocyclers "GeneAmp PCR System 2700" ("Applied Biosystems"), Gradient Palm Cycler ("Corbett Research", "MyCycler" ("BioRad"), "Uno-2" ("Biometra")
- 0,5 ml tubes are used for thermocyclers "Terzik" ("DNA-Technology"), "Uno-2" ("Biometra")

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 *Influenza virus A/H1-swine***.
3. Add above 1 drop of **mineral oil for PCR** (about **25 µl**).
4. Prepare 2 tubes with **PCR-mix-1-FEP/FRT *Influenza virus A/H1-swine*** and mark them as **Background**. Add **17 µl** of **PCR-mix-Background** 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 *Influenza virus A/H1-swine***. Add above **1** drop of **mineral oil for PCR**.
5. Using tips with aerosol filter add under oil or directly on oil **10 µl** of **cDNA samples** obtained at the stage of RNA reverse transcription reaction. cDNA samples should be diluted in accordance with instruction manual for **AmpliSens® *Influenza virus A/B*-FL** or **AmpliSens® *Influenza virus A/H5N1*-FL**.

6. Carry the control amplification reaction:

NCA - add **10 µl** of **TE-buffer** to the tube instead of **cDNA sample**.

C+ - add **10 µl** of **Positive Control cDNA *Influenza virus A/H1-swine* (C+)** to the tube.

CS+ - add **10 µl** of **Positive Control STI (CS+)** to the tube.

7.3.2. Amplification.

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

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

Table 1

Programming thermocycler for Influenza virus A/H1-swine cDNA amplification

Step	Thermocycler with active temperature adjustment:			Thermocyclers with block temperature adjustment:		
	Temperature	Time	Cycles	Temperature	Time	Cycles
0	95 °C	pause		95 °C	pause	
1	95 °C	5 min	1	95 °C	5 min	1
2	95 °C	10 sec	42	95 °C	10 sec	42
	54 °C	20 sec		54 °C	25 sec	
	72 °C	10 sec		72 °C	25 sec	
3	72 °C	1 min	1	72 °C	1 min	1
4	10 °C	storage		10 °C	storage	

8. DATA ANALYSIS.

Detection is conducted on ALA-1 fluorescence detector.



Please read Aladin Operating Manual before use of this kit.

Program the detector according to manufacturer's manual and Appendix 1.

8.2. Results interpretation.

- When the analysis is complete the results are automatically shown in the table as follows:
 - pos** – positive result;
 - neg** – negative result;
 - eq** – equivocal result (signal at the channel for detection of specific cDNA exceed threshold value for negative samples, but does not exceed threshold value for positive samples (signal is in grey zone));
 - nd** – invalid result (specific signal and IC signal does not detect (does not exceed threshold value) in the sample).
- Result of the analysis is considered reliable only if both Positive and Negative Controls of amplification are passed (Table 2).

Table 2

Results for controls

Control	Stage for control	Result of automatic interpretation		Interpretation
		FAM	HEX	
NCA	Amplification	-	nd	OK
C-	RNA extraction and reverse transcription	+	neg	OK
C+	Amplification	-	pos	OK
CS+	Amplification	+	neg	OK

9. TROUBLESHOOTING.

If analysis results are not obtained as per the following examples:

- Preparing the PCR and detection should be repeated for samples with result **nd** (excepting NCA).
In the case of analogous result, it is necessary to repeat the sample analysis, beginning with extraction stage. For the NCA sample the result **nd** is normal.
- Preparing the PCR and detection should be repeated for samples with result **eq**. In the case of analogous result, the samples are considered to be positive.
- No positive signal in positive control of PCR (PC) can indicate incorrect programming of the temperature profile of the thermocycler, incorrect configuration of the PCR reaction, or storage conditions for kit components not complying with manufacturer instruction, or reagent kit has expired. Check programming of the thermocycler (see 7.2.2.), storage conditions, and the expiration date of the reagents and repeat PCR reaction once again for all samples.
- Positive signal in negative controls (C-, NCA) indicates the reagent or sample contamination. In this case results of the analysis are considered invalid. It is necessary to repeat the analysis of all tests, and also to take measures to detect and eliminate the source of contamination.
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® Influenza virus A/H1-swine-FEP** PCR kit are to be stored between 2 to 8 °C. They also must be stable until the expiry date stated on the label.

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of the **AmpliSens® Influenza virus A/H1-swine-FEP** PCR kit is 1x10³ copies per 1 ml of sample (copies/ml).



The claimed analytical features of **AmpliSens® Influenza virus A/H1-swine-FEP** PCR kit are guaranteed only when additional reagents kits «RIBO-sorb» or «RIBO-prep» and «REVERTA-L» (manufactured by Federal State Institution of Science Central Research Institute of Epidemiology) or automated station «NucliSENS® easyMAG™» (manufactured by «bioMérieux», France) in case of RNA isolation are used.

11.2. Specificity.

Specificity of **AmpliSens® Influenza virus A/H1-swine-FEP** PCR kit is ensured by selection of specific primers and probes, as well as the selection of strict reaction conditions. The primers and probes have been checked for possible homologies to all in gene banks published sequences by sequence comparison analysis. Specificity of **AmpliSens® Influenza virus A/H1-swine-FEP** PCR kit was confirmed in laboratory clinical trials.













12. REFERENCES.

1. Manual “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® Influenza virus A/H1-swine-FEP** PCR kit has been 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		Contains sufficient for <n> tests
	Authorised representative in the European Community.		Consult instructions for use
	Caution, consult accompanying documents		Negative Control of Amplification
C+	Positive Control cDNA <i>Influenza virus A/H1-swine</i>	CS+	Positive Control STI
C-	Negative Control of RNA extraction and reverse transcription		