



IVD

For *in Vitro* Diagnostic Use

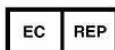
AmpliSens® *Influenza virus A/H1-swine-FRT* PCR kit

Instruction Manual

AmpliSens®

TABLE OF CONTENTS

1. INTENDED USE.....	3
2. PRINCIPLE OF PCR DETECTION.....	3
3. CONTENT.....	3
4. EQUIPMENT ADDITIONALLY REQUIRED.....	4
5. GENERAL PRECAUTIONS.....	4
6. SAMPLING AND HANDLING.....	5
7. PROTOCOL.....	5
8. DATA ANALYSIS.....	7
9. TROUBLESHOOTING.....	7
10. STABILITY AND STORAGE.....	7
11. SPECIFICATIONS.....	7
12. REFERENCES.....	8
13. QUALITY CONTROL.....	8
14. EXPLANATION OF SYMBOLS.....	8



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
www.ecoli.sk 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/H1-swine-FRT* PCR kit is an *in vitro* nucleic acid amplification test for identification of *Influenza virus A/H1-swine* cDNA in clinical material by using real-time hybridization-fluorescence detection.

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 real-time PCR the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product. 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® *Influenza virus A/H1-swine-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® *Influenza virus A/H1-swine-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, which greatly diminishes the frequency of nonspecifically primed reactions. In variant FRT-50 F “hot-start” is guaranteed by application of polymerase (TaqF). Chemically modified polymerase (TaqF) activates by heating at 95°C for 15 min.

3. CONTENT.

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

AmpliSens® *Influenza virus A/H1-swine-FRT* PCR kit variant FRT (for use with RG), [REF](#) R-V55(RG)-CE

AmpliSens® *Influenza virus A/H1-swine-FRT* PCR kit variant FRT-50 F (for use with SC), [REF](#) R-V55-F(SC)-CE

AmpliSens® *Influenza virus A/H1-swine-FRT* PCR kit variant FRT 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
PCR-mix-2-FL	colorless, clear liquid	0.77	1 tube
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 STI-rec during the DNA 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-FRT* PCR kit variant FRT is intended for 55 reactions, including controls.

AmpliSens® *Influenza virus A/H1-swine-FRT* PCR kit variant FRT-50 F includes:

Reagent	Description	Volume (ml)	Amount
PCR-mix-1-FEP/FRT (F) <i>Influenza virus A/H1-swine</i>	colorless, clear fluid	0,12	5 tubes
PCR-mix-2-FRT	colorless, clear fluid	0,3	1 tube
Polymerase (TaqF)	colorless, clear fluid	0,03	1 tube
Positive Control cDNA <i>Influenza virus A/H1-swine</i> (C+)	colorless, clear fluid	0,1	1 tube
Positive Control STI (CS+)	colorless, clear fluid	0,1	1 tube
TE-buffer	colorless, clear fluid	0,5	1 tube
Negative Control (C-)*	colorless, clear fluid	1,2	1 tube
Internal Control STI-rec (IC)**	colorless, clear fluid	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 DNA 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*-FRT PCR kit variant FRT-50 F 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
- Rotor-Gene™ 3000 or Rotor-Gene™ 6000 (Corbett Research) Instrument; SmartCycler II (Cepheid, USA) Instrument
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity
- 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 and eye protection when handling specimens and reagents. 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 specimens and unused reagents in accordance with local regulations.
- Specimens 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 suitable disinfectant.
- Avoid sample or reagent contact with the skin, eyes and mucose membranes. If any of these solutions come into contact, rinse immediately with water and seek medical advice immediately.
- 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.
- Workflow in the laboratory must proceed in a one directional manner, beginning in the Extraction Area and moving 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*-FRT PCR kit is intended for analysis of cDNA samples, in which the RNA *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 following nucleic acid extraction kits for RNA isolation:

- "RIBO-sorb", [REF](#) K2-1-Et-50-CE.
- "RIBO-prep", [REF](#) K2-9-Et-100-CE.

Automatic device «NucliSENS® easyMAG™» can be used either.



Carry the RNA isolation according to the manufacturer instruction.

7.2. Reverse transcription

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

- REVERTA-L, [REF](#) K3-4-50-CE, which contains RT-G-mix-1.
- REVERTA-L, [REF](#) K3-4-100-CE, which contains RT-G-mix-1.



Carry the reverse transcription procedure according to the manufacturer instruction.

7.3. Preparing the PCR.

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

7.3.1 Preparing tubes for PCR.

Variant FRT

1. Prepare 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.

2. Add **7 µl** of **PCR-mix-2-FL** to the surface of wax layer of each tube, that it does not fall under the wax and mix with **PCR-mix -1-FEP/FRT *Influenza virus A/H1-swine***.

Variant FRT-50F

1. Prepare required number of tubes with **PCR-mix-1-FEP/FRT (F) *Influenza virus A/H1-swine*** (1 tube is intended for 11 reactions). Vortex the tube, then centrifuge shortly.

2. For carrying of N reactions mix in a new tube: **10*(N+1) µl** of **PCR-mix-1-FEP/FRT (F) *Influenza virus A/H1-swine***, **5.0*(N+1) µl** of **PCR-mix-2-FRT** and **0.5*(N+1) µl** of **polymerase (TaqF)**. Vortex the tube, then centrifuge shortly. Transfer **15 µl** of prepared mix into 0,025 ml microtubes.

Steps 3 and 4 are applied for both variants.

3. Using tips with aerosol filter add 10 µl of cDNA samples obtained at the stage of RNA reverse transcription reaction after preliminary dilution in compliance with instruction manuals for **AmpliSens® *Influenza virus A/B-FL*** or **AmpliSens® *Influenza virus A/H5N1-FL***.

4. Carry out **control amplification reactions**:

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

C+ add **10 µl** of **PC cDNA *Influenza virus A/H1-swine*** to the tube labeled C+.

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

7.3.2. Amplification

7.3.2.1. RG

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:

a.	Hold	95 °C – 5 min*
b.	Cycling	95 °C – 10 sec
		54 °C – 20 sec
		72 °C – 10 sec
		Cycles repeats – 10 times
c.	Cycling 2	95 °C – 10 sec
		54 °C – 20 sec - Detection
		72 °C – 10 sec
		Cycles repeats – 35 times

* 15 min for variant FRT-50F

Fluorescence detection is on the 2-nd pass (**54°C**) in FAM/Green and JOE/Yellow fluorometer channels.

7.3.2.2. SC



This program is used in the case of AmpliSens® *Influenza virus A/H1-swine-FRT* PCR kit variant FRT-50 F

1. Program the «SmartCycler II» («Cepheid», USA) according to manufacturer's manual and Appendix 2.

2. Create a temperature profile on your «SmartCycler II» («Cepheid», USA) instrument as follows:

- | | | | |
|----|--------|---------------------|--|
| 1. | Stage1 | Hold | 95 °C – 900 c |
| 2. | Stage2 | 2-Temperature Cycle | 95 °C – 15 c
54 °C – 25 c
72 °C – 25 c |

Repeat – 42

Fluorescence detection is on the 2-nd pass (54°C) in FAM and Cy3 fluorometer channels.

8. DATA ANALYSIS.

The results are interpreted by the device software by the crossing (or not) of the fluorescence curve with the threshold line.

Results are accepted as relevant if both positive and negative controls of amplification along with negative control of extraction are passed (see table 1).

Table 1 Results for controls

Control	PCR stage for control	Ct channel		Interpretation
		FAM/Green	JOE/Yellow/ Cy3	
C-	RNA isolation	Pos (<Z*)	Neg	OK
NCA	PCR	Neg	Neg	OK
C+	PCR	Neg	Pos (< X*)	OK
CS+	PCR	Pos (<Y*)	Neg	OK

* For X, Y, Z values see Appendix 1 and 2.

For results interpretation see Appendix 1 and 2.

9. TROUBLESHOOTING

1. If no signal is detected for Positive Controls of amplification, it can suggest incorrect programming of the temperature profile, 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 devices, storage conditions, and the expiration date of the reagents should be checked, and then the PCR should be repeated.

2. If the signal is registered in Negative Control of amplification (NCA) in any of the channels, it indicates the contamination of reagents or samples. In this case results of the analysis for all samples 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-FRT* PCR kit (except for Polymerase (TaqF), PCR-mix-1-FEP/FRT (F) *Influenza virus A/H1-swine* and PCR-mix-2-FRT) are to be stored at 2-8 °C, when not in use. All components of the PCR kit must be stable until the expiry date stated on the label.



Polymerase(TaqF), PCR-mix-1-FEP/FRT (F) *Influenza virus A/H1-swine* and PCR-mix-2-FRT are to be stored at not more than – 16°C.

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of the AmpliSens® *Influenza virus A/H1-swine-FRT* PCR kit is 1×10^3 copies per 1 ml of sample (copies/ml).



Claimed analytical features of AmpliSens® *Influenza virus A/H1-swine-FRT* PCR kit are guaranteed only when additional reagents kits «RIBO-sorb» or «RIBO-prep» and «REVERTA-L» (ILS) or automated station «NucliSENS® easyMAG™» (manufactured by «bioMérieux», France) for RNA isolation are used

11.2. Specificity.

Specificity of AmpliSens® *Influenza virus A/H1-swine-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® *Influenza virus A/H1-swine-FRT* PCR kit was confirmed in laboratory clinical trials.

12. REFERENCES.

1. 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® *Influenza virus A/H1-swine-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