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For Professional Use Only

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

## Instruction Manual

**AmpliSens®**

EC REP

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## 1. INTENDED USE

**AmpliSens®** ***Influenza virus*** **A/H1-swine-FRT** PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Influenza virus* A/H1N1(sw2009) RNA in clinical materials (nasal and oropharyngeal swabs, sputum or nasopharyngeal and tracheal aspirates, and autopsy material (fragments of affected parts of lungs)) by using real-time hybridization-fluorescence detection.

It is recommended to combine nasal and oropharyngeal swabs in one tube. To do this, after sampling, place the working ends of probes into one tube with 500 µl of Transport Medium for Storage and Transportation of Respiratory Swabs and analyze as one sample.

The results of PCR analysis are taken into account in complex diagnostics of disease.

## 2. PRINCIPLE OF PCR DETECTION

*Influenza virus* A/H1N1(sw2009) detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *Influenza virus* A/H1N1(sw2009) 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 during thermocycling. The real-time PCR monitoring of 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 that contains the Internal Control (IC). It must be used in the extraction procedure in order to control the extraction 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 the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using a wax layer. Wax melts and reaction components mix only at 95 °C.

## 3. CONTENT

**AmpliSens®** ***Influenza virus*** **A/H1-swine-FRT** PCR kit is produced in 4 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 (for use with iQ) **REF** R-V55(iQ)-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-50 F (for use with RG, iQ, Dt, SC)

**REF** R-V55-F(RG,iQ,Dt,SC)-CE.

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

| ***Reagent*** | ***Description*** | ***Volume (ml)*** | ***Quantity*** |
|---|---|---|---|
| **PCR-mix-1-FEP/FRT *Influenza virus* A/H1-swine** (ready-to-use single-dose test tubes (*under wax)*) | 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 *Influenza virus* A/H1-swine ($C+_{A/H1\text{-}swine}$)** | colorless clear liquid | 0.1 | 1 tube |
| **Positive Control STI-88 (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 extraction 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® *Influenza virus* A/H1-swine-FRT PCR kit 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)*** | ***Quantity*** |
|---|---|---|---|
| **PCR-mix-1-FEP/FRT (F) *Influenza virus* A/H1-swine** | colorless clear liquid | 0.12 | 5 tubes |
| **PCR-mix-2-FRT** | colorless clear liquid | 0.3 | 1 tube |
| **Polymerase (TaqF)** | colorless clear liquid | 0.03 | 1 tube |
| **Positive Control cDNA *Influenza virus* A/H1-swine ($C+_{A/H1\text{-}swine}$)** | colorless clear liquid | 0.1 | 1 tube |
| **Positive Control STI-88 (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-50 F is intended for 55 reactions (including controls).

## 4. ADDITIONAL REQUIREMENTS

- RNA extraction kit.
- NucliSENS easyMAG automated system for DNA/RNA extraction (BioMérieux, France).
- Reagent kit to NucliSENS easyMAG automated system (NucliSens buffer for extraction 1, NucliSens buffer for extraction 2, NucliSens buffer for extraction 3, NucliSens lysis buffer, NucliSens magnetic silica) (BioMérieux, France).
- Reverse transcription kit.
- Transport medium.
- 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 a rotor for 2-ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia); Rotor-Gene Q (Qiagen, Germany), Mx3000P (Stratagene, USA), Mx3000 (Stratagene, USA), SmartCycler II (Cepheid, USA), iCycler iQ or iQ5 (Bio-Rad, USA), or equivalent).
- Disposable polypropylene microtubes for PCR (0.1- or 0.2-ml; for example, Axygen, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –16 °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 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 accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant, such as 0.5% sodium hypochlorite or another suitable disinfectant.
- Avoid contact with the skin, eyes, and mucosa. If skin, eyes, or mucosa contact, immediately flush with water and seek medical attention.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- The laboratory process must be one-directional, it should begin in the Extraction Area and then 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 the 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 the analysis of RNA extracted by RNA extraction kits from nasal and oropharyngeal swabs; sputum (or nasopharyngeal and tracheal aspirates), autopsy material (fragments of affected parts of lungs).

**PREPARATION OF CLINICAL MATERIAL FOR RNA EXTRACTION**

**Swabs** are used without pretreatment.

**Sputum or tracheal aspirate.** Use reagent Mucolysin (REF 180-CE) manufactured by FBIS CRIE for sputum pretreatment. See the instruction manual to Mucolysin for a proper use. The pretreated sputum (100 µl) is used for RNA extraction. If it is necessary to repeat the test, the rest of sputum can be frozen.

**Autopsy material** is homogenized using sterile porcelain mortars and pestles. Then, prepare a 10 % suspension in a sterile saline or phosphate buffer. Transfer the suspension to a 1.5-ml tube and centrifuge at 10,000 rpm for 5 min. The supernatant (100 µl) is used for RNA extraction. If it is necessary to repeat the test, the remaining sputum can be frozen.

## 7. WORKING CONDITIONS

**AmpliSens® *Influenza virus* A/H1-swine-FRT** PCR kit should be used at 18–25 °C.

## 8. PROTOCOL

### 8.1. RNA extraction

It is 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.

Extract DNA according to the manufacturer's instructions.

The volume of Internal Control STI-87-rec (IC) is 10 µl.

- NucliSENS easyMAG automated system can be used.

  If RNA is extracted using the NucliSENS easyMAG automated system:
  - EM-plus kit REF K2-15-96-CE (manufactured by FBIS CRIE) must be used
  - Add 30 ml (the whole content of the bottle) of the **RT-G component from the EM-Plus kit** to the bottle with the NucliSens lysis buffer, close tightly the cap and **carefully** mix by turning upside down 7-10 times (this procedure is performed once for each reagent kit).

  - Mix 10 µl of the **Internal Control (IC) sample with** 10 µl of **NucliSens magnetic silica** and 10 µl of **Component A** from the **EM-plus kit** with per one sample for RNA/DNA isolation in a new sterile tube using disposable tips with aerosol barriers.
  - Set a sample volume as 0.1 ml or 1 ml
  - Set the eluate volume as 50–60 µl (up to 100 µl)
  - Both *On-board* and *Off-board* Lysis Buffer Dispensing and Lysis Incubation

modes can be used

### 8.2. Reverse transcription

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

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

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

### 8.3. Preparing PCR

#### 8.3.1. Preparing tubes for PCR

**Variant FRT**

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.
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**.

**Variant FRT-50 F**

1. Thaw the tubes with **PCR-mix-1-FEP/FRT (F) *Influenza virus* A/H1-swine, PCR-mix-2-FRT,** and **polymerase (TaqF)**. Vortex the tubes, then centrifuge briefly.
   Take the required number of the tubes/stripes for amplification of cDNA obtained from clinical and control samples.
2. For N reactions, add to 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;**
   0.5*(N+1) µl of **polymerase (TaqF).**
   Vortex the tube, then centrifuge briefly. Transfer **15 µl** of the prepared mixture to each tube.

Steps 3 and 4 are applied for both variants.

3. Add **10 µl** of c**DNA** obtained from clinical or control samples at the RNA reverse transcription stage into the prepared tubes using tips with aerosol barrier.
4. Carry out 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 *Influenza virus* A/H1-swine** to the tube labeled C+ (Positive Control of Amplification).

**CS+** - Add **10 µl** of **Positive Control STI-88** to the tube labeled CS+ (Positive Control of Amplification of IC)

**C–** - Add **10 µl** of the sample extracted from **Negative Control** to the tube labeled C- (Negative control of extraction).

### 8.3.2. Amplification

Program the real-time amplification instrument according to manufacturer's manual and Guidelines [2].

1. Create a temperature profile on your instrument as follows:

***Influenza virus* A/H1N1(sw2009) cDNA amplification program**

| | Rotor-type instruments[1] | | | Plate-type instruments[2] | | |
|---|---|---|---|---|---|---|
| **Step** | ***Temperature, °C*** | ***Time*** | **Cycles** | ***Temperature, °C*** | ***Time*** | **Cycles** |
| 1 | **95** | **5 min (variant FRT)**<br>**15 min (variant FRT-50 F)** | 1 | **95** | **5 min (variant FRT)**<br>**15 min (variant FRT-50 F)** | 1 |
| 2 | **95** | 10 s | 10 | **95** | 10 s | 10 |
| | **54** | 20 s | | **54** | 25 s | |
| | **72** | 10 s | | **72** | 25 s | |
| 3 | **95** | 10 s | 35 | **95** | 10 s | 35 |
| | **54** | 20 s<br>*fluorescent signal detection* | | **54** | 25 s<br>*fluorescent signal detection* | |
| | **72** | 10 s | | **72** | 25 s | |

Fluorescent signal is detected in the channels designed for the FAM/Green and JOE/Yellow/HEX fluorophores on the 2nd step (60 °C) of stage 3 (other channels are enabled if several tests are simultaneously carried out in a single run).

2. Adjust the fluorescence channel sensitivity according to *Important Product Information Bulletin.*
3. Insert tubes into the reaction module of the device.
4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

## 9. DATA ANALYSIS

*Influenza virus* A/H1N1(sw2009) RNA is detected in the JOE/Yellow/HEX/Cy3 fluorescence channel, Internal Control STI-rec cDNA is detected in the FAM/Green fluorescence channel.

See the **Manufacturer's manual, Guidelines** and **Important product information bulletin** for data analysis settings.

### Interpretation of results

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

[1] For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q or equivalent.
[2] For example, Mx3000P, Mx3000, iCycler iQ, iQ5, or equivalent.

- *Influenza virus* A/H1N1(sw2009) RNA is **detected** in a sample if its Ct value is detected in the results grid in the JOE/Yellow/HEX/Cy3 channel and if it is not greater than the Ct value indicated in the Important Product Information Bulletin.
- *Influenza virus* A/H1N1(sw2009) RNA is **not detected** if its Ct value is not detected in the results grid (the fluorescence curve does not cross the threshold line) in the JOE/Yellow/HEX/Cy3 channel and the Ct value in the results grid in the FAM/Green channel is detected and does not exceed the boundary Ct value.
- The result of analysis is **invalid** if the Ct value is not detected in the results grid (the fluorescence curve does not cross the threshold line) in the JOE/Yellow/HEX/Cy3 channel and the Ct value in the results grid in the FAM/Green channel is not detected or exceeds the boundary Ct value. In such cases PCR should be repeated.

The results of analysis are considered reliable only if the results obtained for both Positive and Negative Controls of amplification and Negative Control of extraction are correct.

**Results for controls**

| Control | Stage for control | Ct value in channel | | Interpretation |
|---|---|---|---|---|
| | | FAM/Green | JOE/Yellow/Cy3 | |
| **C–** | DNA extraction | < boundary value* | Neg | OK |
| **NCA** | Amplification | Neg | Neg | OK |
| **C+** | Amplification | Neg | < boundary value | OK |
| **CS+** | Amplification | < boundary value | Neg | OK |

*For boundary Ct values, see the Important Product Information Bulletin.

## 10. TROUBLESHOOTING

Results of analysis are not taken into account in the following cases:

1. If the Ct value in the JOE/Yellow/HEX/Cy3 channel is higher than the specified boundary Ct value, PCR should be repeated. If the same result is obtained, repeat analysis starting from the extraction stage.
2. If a Ct value is present for C– in the JOE/Yellow/HEX/Cy3 channel and for NCA in the FAM/Green and JOE/Yellow/HEX/Cy3 channels in the results grid, this indicates contamination of reagents or samples. In such cases, the results of analysis must be considered as invalid. Repeat analysis and take measures to detect and eliminate the source of contamination.
3. If no signal is detected for the positive controls of amplification, this may suggest that the programming of the temperature profile of the used Instrument was incorrect, or that the configuration of the PCR reaction was incorrect, or that the storage conditions for kit components did not comply with the manufacturer's instruction, or that the reagent kit has expired. Check programming of the used instrument, storage conditions, and the expiration date of the reagents and then repeat PCR.

If you have any further questions or if you encounter problems, please contact our Authorized representative in the European Community.

## 11. TRANSPORTATION

**AmpliSens® *Influenza virus* A/H1-swine-FRT** PCR kit should be transported at 2–8 ºC for no longer than 5 days.

## 12. STABILITY AND STORAGE

All components of the **AmpliSens® *Influenza virus* A/H1-swine-FRT** PCR kit are to be stored at 2–8 °C (except for PCR-mix-1-FEP/FRT (F) *Influenza virus* A/H1-swine, PCR-mix-2-FRT, and polymerase (TaqF)) when not in use.

All components of the **AmpliSens® *Influenza virus* A/H1-swine-FRT** PCR kit are stable until the expiration date on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

PCR-mix-1-FEP/FRT (F) *Influenza virus* A/H1-swine, PCR-mix-2-FRT, and polymerase (TaqF) are to be stored at temperature from minus 24 to minus 16 °C when not in use.

PCR-mix-1-FEP/FRT *Influenza virus* A/H1-swine and PCR-mix-1-FEP/FRT (F) *Influenza virus* A/H1-swine are to be kept away from light.

## 13. SPECIFICATIONS

### 13.1. Sensitivity

The analytical sensitivity of **AmpliSens® *Influenza virus* A/H1-swine-FRT** PCR kit is following:

| Clinical material | Nucleic acid extraction kit | Sensitivity, copies/ml |
|---|---|---|
| Nasal and oropharyngeal swabs [3] | RIBO-prep | $1x10^3$ |
| | RIBO-sorb | $5x10^3$ |

### 13.2. Specificity

The analytical specificity of **AmpliSens® *Influenza virus* A/H1-swine-FRT** PCR kit is ensured by selection of specific primers and probes as well as strict reaction conditions. The primers and probes were checked for possible homologies to all sequences deposited

[3] Nasal and oropharyngeal swabs should be placed into the Transport Medium for Storage and Transportation of Respiratory Swabs (**REF** 957-CE, 958-CE, 959-CE).

in gene banks by sequence comparison analysis. The clinical specificity of **AmpliSens®** ***Influenza virus*** **A/H1-swine-FRT** PCR kit was confirmed in laboratory clinical trials.

**AmpliSens®** ***Influenza virus*** **A/H1-swine-FRT** PCR kit determines the fragment of the *Influenza virus* A/H1N1 hemagglutinin gene, which is related to the North American strain of swine *Influenza virus*. The specific activity of the PCR kit was confirmed by analyzing A/California/04/2009(H1N1) and A/California/07/2009(H1N1) isolates provided by CDC.

Nonspecific reactions were absent is tests of 26 reference strains and isolates of epidemic *Influenza viruses* A/H1N1 isolated in 1977 to 2008 in the Russian Federation, Ukraine, and Belarus Republics; *Influenza virus* A subtypes H13N2, H9N2, H8N4, H2N3, H2N9, H3N2, H3N8, H4N6, H11N6, H12N5, H1N1, H6N2, H10N7, H5N3, H7N1, H5N2, H5N3, and H2N2; *Influenza virus* B strains Yamagata and Victoria; as well as strains and isolates of the main pathogens causing human acute respiratory infections; and nucleic acids of the human genome.

## 14. REFERENCES


1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics”, developed by Federal State Institute of Science “Central Research Institute of Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2008.
2. Guidelines “Real-Time PCR Detection of *Influenza virus* A/H1N1(sw2009) RNA”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow.


## 15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science “Central Research Institute for 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.

## 16. KEY TO SYMBOLS USED

| | | | |
|---|---|---|---|
| REF | Catalogue number |  | Caution |
| LOT | Batch code |  | Sufficient for |
| IVD | *In vitro* diagnostic medical device |  | Expiration Date |
| VER | Version |  | Consult instructions for use |
|  | Temperature limitation |  | Keep away from sunlight |
|  | Manufacturer | **NCA** | Negative control of amplification |
|  | Date of manufacture | **C–** | Negative control of extraction |
| EC REP | Authorised representative in the European Community | **C+** | Positive control of amplification |
| **CS+** | Positive Control of Amplification of IC | **IC** | Internal control |
| **FBIS CRIE** | Federal Budget Institute of Science “Central Research Institute for Epidemiology” | **PCE** | Positive control of extraction |

**List of Changes Made in the Instruction Manual**

| VER | Location of changes | Essence of changes |
|---|---|---|
| 24.01.11 | Reference number | Reference numbers R-V55(iQ)-CE and R-V55-F(RG,iQ,Dt,SC)-CE are added |
| | Cover page | The phrase “For Professional Use Only” was added |
| | Content | New sections “Working Conditions” and “Transportation” were added |
| | | The “Explanation of Symbols” section was renamed to “Key to Symbols Used” |
| | Stability and Storage | The information about the shelf life of reagents before and after the first use was added |
| | | Information that PCR-mix-1-FEP/FRT *Influenza virus* A/H1-swine and PCR-mix-1-FEP/FRT (F) *Influenza virus* A/H1-swine are to be kept away from light was added |
| | Key to Symbols Used | The explanation of symbols was corrected |
| 23.06.11 RT | Cover page, text | The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology” |
| 24.07.12 lvl | Content | AmpliSens® *Influenza virus* A/H1-swine-FRT PCR kit variant FRT-50 F (for use with SC) was added |
| | Footer | A new catalogue number was added |