RUO

For Professional Use Only

AmpliSens® *Influenza virus* A/B-FRT

PCR kit

Instruction Manual

AmpliSens®

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1. INTENDED USE

AmpliSens® ***Influenza virus*** **A/B-FRT** 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 and oropharyngeal swabs; sputum or nasopharyngeal or tracheal aspirates; and autopsy material) by using real-time hybridization-fluorescence detection of amplified products.

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

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 the pathogen genome specific region using specific ***Influenza virus*** **A and B** 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 fluorescence intensities' monitoring during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR finished. **AmpliSens®** ***Influenza virus*** **A/B-FRT** PCR kit is a qualitative test which contains the Internal Control (IC) obligatory used in the extraction procedure in order to control the separating process of each individual sample and to identify possible reaction inhibitors. **AmpliSens® Influenza virus A/B-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 a wax layer or a chemically modified polymerase (TaqF). Wax melts and reaction components mix only at 95 ºC. The chemically modified polymerase (TaqF) is activated by heating at 95 ºC for 15 min.

3. CONTENT

AmpliSens® ***Influenza virus*** **A/B-FRT** PCR kit is produced in 1 form:

AmpliSens® ***Influenza virus*** **A/B-FRT** variant FRT-50 F PCR kit, **REF** R-V36-F(SC)-CE.

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

Reagent	***Description***	***Volume (ml)***	***Quantity***
PCR-mix-1-FEP/FRT (F) ***Influenza virus*** **A/B**	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 ($C+_A$)**	colorless clear liquid	0.1	1 tube
Positive Control cDNA ***Influenza virus*** **B ($C+_B$)**	colorless clear liquid	0.1	1 tube

Positive Control STI (CS+)	colorless clear liquid	0.1	1tube
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 during the RNA extraction procedure directly to the sample/lysis mixture.

AmpliSens® ***Influenza virus*** **A/B-FRT** PCR kit is intended for 55 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- RNA/DNA extraction kit.
- Reverse transcription kit.
- Disposable powder-free gloves.
- 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 thermocycler SmartCycler II (Cepheid, USA) or equivalent).
- Disposable polypropylene microtubes for PCR (0.5- or 0.2-ml; for instance, Axygen, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer at ≤ –16 °C.
- Reservoir 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 all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use protective gloves and laboratory cloths, and protect eye 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 specimens and unused reagents in accordance with local regulations.

• Samples should be considered potentially infectious and handled in biological cabinet compliance with appropriate biosafety practices.

• Clean and disinfect all specimens or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.

• Avoid specimens and reagents contact with the skin, eyes, and mucous membranes. If 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 DNA amplification techniques.

• Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where 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 are described in the manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® ***Influenza virus*** **A/B-FRT** PCR kit is intended to analyze RNA extracted with RNA extraction kits from in the clinical material (nasal, throat swabs; sputum or aspirate of nasopharynx or trachea; autopsy material).

For trachea sputum and aspirate pretreatment please use Mucolysin reagent **REF** 180-CE.

7. WORKING CONDITIONS

AmpliSens® ***Influenza virus*** **A/B-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-100-CE;

- NucliSENS easyMAG automated system.

Please carry out the RNA extraction according to the manufacturer protocol.
The volume of clinical sample is 100 µl.
The volume of Internal Control STI-rec (IC) is 10 µl.

Using the NucliSENS easyMAG automated system set the sample volume as 0,1 ml and eluate volume as 25 µl. Both *On-board* and *Off-board* Lysis Buffer Dispensing and Lysis incubation are possible. *Off-board* extraction is preferred for clot containing samples (aspirates and sputum). In case of *Off-board* operating, add 550 µl of Lysis buffer into each sample tube.

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.

8.3. Preparing PCR

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

Variant FRT-50 F

1. Thaw the tubes with **PCR-mix-1-FEP/FRT (F) *Influenza virus* A/B, PCR-mix-2-FRT, and polymerase (TaqF)**. Vortex the tubes**,** then centrifuge briefly.

   Take the required number of the tubes/strips 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/B,

   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 required in 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).

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

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

CS+ -Add **10 µl** of **Positive Control STI** to the tube labeled CS+.

8.3.2. Amplification

If you use **SmartCycler II** for detection, run the following program on the thermocycler.

1. Stage 1	Hold	95 °C – 900 s	
2. Stage 2	Temperature Cycle	95 °C – 15 s	
		54 °C – 25 s – Detection	

72 °C – 25 s

Repeat – 42 times.

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

9. DATA ANALYSIS

The analysis results are considered to be valid, only if the control samples results comply with the following (see Tables 1).

Table 1

Results for controls (SmartCycler II)

Control	Stage for control	Ct value in channel		
		FAM(Green)/ FAM	JOE(Yellow)/ JOE/Cy3	ROX(Orange)/ROX/ Texas Red
C-	RNA extraction and reverse transcription	positive (< N)	negative	negative
NCA	PCR	negative	negative	negative
$C+_A$	PCR	negative	negative	positive (< X)
$C+_B$	PCR	negative	positive (< Y)	negative
CS+	PCR	positive (< Z)	negative	negative

For N, X, Y, Z values see Guidelines.

1. The sample is considered to be positive for *Influenza virus* A if its Ct value is less than X on ROX/Orange channel. If Ct value is more than X PCR should be repeated and the sample is considered to be positive in case of result's repeat or in case of result is less than X.
2. The sample is considered to be positive for *Influenza virus* B if its Ct value is less than Y on JOE/Yellow channel If Ct value is more than Y PCR should be repeated and the sample is considered to be positive in case of result's repeat or in case of result is less than Y.
3. The sample is considered to be negative if the Ct values are absent on ROX/Orange and JOE/Yellow channels and the Ct value is less than Z on FAM/Green channel.

10. TROUBLESHOOTING

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

- Positive signal absence in $C+_A$ and/or $C+_B$ and/or CS+ samples indicates the incorrect programming of the temperature profile of the thermocycler, incorrect configuration of the PCR reaction, or kit storage conditions did not comply with manufacturer instruction, or reagents kit has expired. It is necessary to check programming of the thermocycler (see 8.3.2.), storage conditions, and the expiration date of the reagents and repeat PCR reaction once again for all samples.
- The presence of positive signal in negative controls (except for C– in FAM) indicates contamination of the reagents or samples. In such case, analysis results must be considered as invalid. Test must be repeated and measures for detecting of contamination source must be taken.
- If the Ct for Internal Control in FAM exceeds N, it indicates an error in RNA extraction. This sample should be examined repeatedly (extraction and amplification with detection).

11. TRANSPORTATION

AmpliSens® *Influenza virus* A/B-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/B-FRT** PCR kit (except for PCR-mix-1-FEP/FRT (F) *Influenza virus* A/B, polymerase(TaqF) and PCR-mix-2-FRT) are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® *Influenza virus* A/B-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/B, polymerase(TaqF), and PCR-mix-2-FRT are to be stored at temperature from minus 24 to minus 16 °C when not in use.

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

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of **AmpliSens® *Influenza virus* A/B-FRT** PCR kit is no less than $1x10^4$ copies per 1 ml of sample (cop/ml).

Claimed analytical features of **AmpliSens® *Influenza virus* A/B-FRT** PCR kit are guaranteed only when additional reagents kits, RIBO-sorb, RIBO-prep, NucliSENS easyMAG automated system and REVERTA-L are used.

13.2. Specificity

The analytical specificity of AmpliSens® Influenza virus A/B-FRT PCR kit is ensured by selection of specific primers and probes, as well as the selection of stringent reaction conditions. The primers and probes were checked for possible homologies to all in gene banks published sequences by sequence comparison analysis. The clinical specificity of AmpliSens® Influenza virus A/B-FRT PCR kit was confirmed in laboratory clinical tests.

14. REFERENCES

1. Handbook “Sampling, transportation, and storage of clinical material for PCR diagnostics”, 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, 2008.

15. QUALITY CONTROL

In accordance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of **AmpliSens® *Influenza virus* A/B-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
RUO	Research Use Only		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
IC	Internal control	**$C+_A$, $C+_B$, CS+**	Positive control of amplification

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
18.03.13 FN	Cover page, Key to symbols used	Symbol IVD was replaced with the symbol RUO