



IVD

For *in Vitro* Diagnostic Use

AmpliSens® Influenza virus A/B-FRT PCR kit

Instruction Manual

AmpliSens®

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1. INTENDED USE.

AmpliSens® *Influenza virus A/B-FEP* 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 real-time hybridization-fluorescence detection of amplified products.

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 via 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 isolation 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 wax layer or chemically modified polymerase (TaqF). The wax melting and reaction mix component occurs only at 95°C. Chemically modified polymerase (TaqF) activates by heating at 95°C for 15 min.

3. CONTENT.

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

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

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 PCR kit includes:

Reagent	Description	Volume (ml)	Quantity
PCR-mix-1-FEP/FRT <i>Influenza virus A/B</i> 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 <i>Influenza virus A</i> (C _A +))	colorless, clear liquid	0.1	1 tube
Positive Control cDNA <i>Influenza virus B</i> (C _B +))	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.

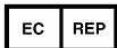
** add 10 µl of Internal Control during the RNA isolation procedure directly to the sample/lysis mixture

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

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

Reagent	Description	Volume (ml)	Quantity
PCR-mix-1-FEP/FRT (F) <i>Influenza virus A/B</i>	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 <i>Influenza virus A</i> (C _A +))	colorless, clear liquid	0,1	1 tube
Positive Control cDNA <i>Influenza virus B</i> (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 isolation procedure as Negative Control of Extraction.



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** add 10 µl of Internal Control during the RNA isolation 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 isolation kit
- Reverse transcription kit
- Disposable powder-free gloves
- Pipettes (adjustable)
- Sterile 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, «Rotor-Gene» 3000/6000(Corbett Research, Australia) or equivalent)
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity.
- Refrigerator for 2–8 °C.
- Deep-freezer with temperature no less than –16°C.
- Reservoir for used tips.

5. GENERAL PRECAUTIONS.

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters 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, laboratory cloths protect eye 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 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 other suitable disinfectant.
 - Avoid specimens and reagents contact with the skin, eyes and mucose 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 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 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.



In detail, sampling 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/B-FRT* PCR kit is intended to analyze RNA extracted with RNA isolation 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.

7. PROTOCOL.

7.1. RNA Isolation.

It's 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 isolation 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* isolation 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.

7.2. Reverse transcription

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

- “REVERTA-L”, [REF] K3-4-50-CE.

7.3. Preparing the PCR.

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

7.3.1.1 Preparing tubes for PCR (in case of using «Rotor-Gene» 3000/6000» or «iQ iCycler» or «iQ5»).

1. Prepare the required quantity of tubes with **PCR-mix-1-FEP/FRT *Influenza virus A/B*** and wax for amplification of studied and control samples cDNA.
2. Add **7 µl** of **PCR-mix-2-FL** to the surface of wax layer of each tube, so that it won't fall under the wax and mix with PCR-mix-1-FEP/FRT *Influenza virus A/B*.
3. Using tips with aerosol barrier add **10 µl** of clinical or control samples **cDNA samples**.

7.3.1.2 Preparing tubes for PCR (in case of using «SmartCycler II»).

1. Prepare the required quantity of tubes with **PCR-mix-1-FEP/FRT (F) *Influenza virus A/B*** (1 tube is intended for 11 reactions), mix and sediment drops from walls of tubes by short vortex .
2. Mix **10*(N+1) µl** of **PCR-mix-1-FEP/FRT (F) *Influenza virus A/B***, **5*(N+1) µl** of **PCR-mix-2-FRT** и **0,5*(N+1) µl** of **Polymerase (TaqF)** in one tube for N reactions .
3. Mix and sediment drops from walls of tubes by short vortex. Add **15 µl** into 0,025 ml tubes.
4. Using tips with aerosol barrier add **10 µl** of clinical or control samples **cDNA samples**.

7.3.1.3. Performing of control amplification reactions.

Perform 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 <i>Influenza virus A</i> to the tube labeled C _A .
C _B	Add 10 µl of Positive Control cDNA <i>Influenza virus B</i> to the tube labeled C _B .
CS+	Add 10 µl of Positive Control STI to the tube labeled CS+.

7.3.2 Amplification.

If you use «**Rotor-Gene» 3000/6000»** for detection then run the following program on the thermocycler.

1. Hold 95 °C - 5 min
2. Cycling 95 °C - 10 s
54 °C - 20 s
72 °C - 10 s
Cycle repeats – 10 times.
3. Cycling 2 95 °C - 10 s
54 °C - 20 s – Detection
72 °C - 10 s
Cycle repeats – 35 times.

If you use «**iQ iCycler» or «iQ5» («BioRad», USA)** for detection then run the following program on the thermocycler

- 95 °C–5 min
10 cycles: 95 °C – 10 s / 54 °C – 25 s / 72 °C – 25 s
35 cycles: 95 °C – 10 s / 54 °C – 25 s (detection) / 72 °C – 25 s

If you use «SmartCycler II» («Cepheid», USA) for detection then run the following program on the thermocycler

1.	Stage1	Hold	95 °C – 900 s
2.	Stage2	2-Temperature Cycle	95 °C – 15 s 54 °C – 25 s 72 °C – 25 s Repeat – 42

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

8. DATA ANALYSIS.

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

Table 1

Control	Stage for control	Ct value on channel		
		FAM (Green)/FAM result	JOE (Yellow)/JOE/Cy3 result	ROX (Orange)/ROX/Texas Red result
C-	RNA isolation 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

- 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.
- 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.
- 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

9. TROUBLESHOOTING.

Results of analysis are not being registered 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 7.3.2.), storage conditions, and the expiration date of the reagents and repeat PCR reaction once again for all samples.
- Positive signal absence on both channels (one for pathogen cDNA detection and the other one for Internal Control detection) indicates the error in RNA isolation process. This sample should be examined repeatedly (isolation, PCR and detection).
- Positive signal presence in negative controls (except for C– in FAM channel) indicates the reagents or samples contamination. In such case analysis results must be considered as irrelevant. Test must be repeated and measures for detecting of contamination source must be undertaken.

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/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 the temperature between 2 °C and 8 °C, when not in use. All components of the **AmpliSens® Influenza virus A/B-FRT** PCR kit are to be stable until labeled expiration date.



PCR-mix-1-FEP/FRT (F) *Influenza virus A/B*, polymerase(TaqF) and PCR-mix-2-FRT are to be stored at the temperature not more than minus 16 °C.

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical sensitivity of **AmpliSens® Influenza virus A/B-FRT** PCR kit is no less than 1x10⁴ 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.

11.2. Specificity.

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. Specificity of **AmpliSens® Influenza virus A/B-FRT** PCR kit was confirmed in laboratory clinical tests.

12. REFERENCES.

- 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 accordance with Federal State Institution of Science "Central Research Institute of 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.

14. SYMBOLS EXPLANATION.



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