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For Professional Use Only

AmpliSens® *Influenza virus* A/B-FEP

PCR kit

Instruction Manual

AmpliSens®

EC REP

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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 end-point 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 pathogen genome specific region using special ***Influenza virus*** **A** and **B** primers. In **F**luorescent **E**nd-**P**oint PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes that bind specifically to the amplified product during thermocycling. A multichannel rotor-type fluorometer is specially designed to detect fluorescent emission from the fluorophores in the reaction mixture after PCR. It allows detection of the accumulating product without re-opening the reaction tubes after the PCR run. **AmpliSens®** ***Influenza virus*** **A/B-FEP** 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/B-FEP** 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/B-FEP** PCR kit is produced in 2 forms:

AmpliSens® ***Influenza virus*** **A/B-FEP** PCR kit (vials 0.5 ml), **REF** V36-50-R0,5-FEP-CE.

AmpliSens® ***Influenza virus*** **A/B-FEP** PCR kit (vials 0.2 ml), **REF** V36-50-R0,2-FEP-CE.

AmpliSens® ***Influenza virus*** **A/B-FEP** PCR kit includes:

Reagent	*Description*	*Volume, ml*	*Quantity*
PCR-mix-1-FEP/FRT *Influenza virus* A/B ready-to-use single-dose test tubes (*under wax)*	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
Mineral oil for PCR	colorless viscous liquid	4.0	1 dropper bottle
PCR-mix-Background	colorless clear liquid	0.5	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	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 during the RNA extraction procedure directly to the sample/lysis mixture (see RIBO-sorb, **REF** K2-1-Et-50-CE protocol or RIBO-prep, **REF** K2-9-Et-50-CE protocol).

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

4. ADDITIONAL REQUIREMENTS

- RNA/DNA extraction kit.
- Reverse transcription kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free 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, Gradient Palm Cycler (Corbett Research, Australia), GeneAmp PCR System 2700 (Applied Biosystems), Uno-2 (Biometra).

- Fluorometer ALA-1/4 (Biosan, Latvia) or equivalent instrument.
- Disposable polypropylene microtubes for PCR (0.5- 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 are described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® ***Influenza virus*** **A/B-FEP** PCR kit is intended for analysis of RNA extracted with RNA/DNA extraction kits from 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-FEP** 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;
- NucliSENS easyMAG automated system.

Carry the RNA extraction according to the manufacturer's protocol.

Add 10 µl of Internal Control STI-rec (IC) into the tubes with clinical samples.

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**.

8.3.1 Preparing tubes for PCR

1. Prepare the required number of the tubes with **PCR-mix-1-FEP/FRT *Influenza virus* A/B** 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 ensuring that it does not fall under the wax and mix with **PCR-mix-1-FEP/FRT *Influenza virus* A/B**.
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/B** and mark them as **Background**. Add **17 µl** of **PCR-mix-Background** to the surface of 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/B**. Add above **1** drop of **mineral oil for PCR.**
5. Using tips with aerosol barrier add **10 µl of cDNA** obtained from clinical or control samples at the RNA reverse transcription stage.
6. 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

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

It is recommended to sediment drops from walls of tubes by short centrifugation (1–3 s) before placing them into the thermocycler.

Table 1

Programming thermocyclers for *Influenza virus* A/B cDNA amplification

	Thermocyclers with active temperature adjustment:						Thermocyclers with block temperature adjustment: **Uno-2 (Biometra)**		
	Terzik (DNA-Technology)			**GeneAmp PCR System 2700 (Applied Biosystems), MaxyGene (Axygen Scientific) Gradient Palm Cycler (Corbett Research), MyCycler (Bio-Rad),**					
Step	Tempe-rature	Time	Cycles	Tempe-rature	Time	Cycles	Tempe-rature	Time	Cycles
0	**95 °C**	pause		**95 °C**	pause		**95 °C**	pause	
1	**95 °C**	5 min	1	**95 °C**	5 min	1	**95 °C**	5 min	1
2	**95 °C**	10 s	42	**95 °C**	10 s	42	**95 °C**	25 s	42
	54 °C	20 s		**54 °C**	25 s		**54 °C**	40 s	
	72 °C	10 s		**72 °C**	25 s		**72 °C**	25 s	
3	**72 °C**	1 min	1	**72 °C**	1 min	1	**72 °C**	1 min	1
4	**10 °C**	storage		**10 °C**	storage		**10 °C**	storage	

9. DATA ANALYSIS

Fluorescence is detected in ALA-1/4 florescence detector.

Please read the ALA-1/4 Operating Manual before use of this kit.

Program the detector according to manufacturer's manual and Guidelines.

9.1. Results interpretation

1. 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 is in grey zone);

   nd – invalid result (specific signal and IC signal are absent in the sample).

2. The result of analysis is considered reliable only if the results obtained for Positive and Negative Controls of Amplification and Negative Control of Extraction are correct (Table 2).

Table 2

Results for controls

Control	Stage for control	Result of automatic interpretation			Interpretation
		FAM (IC)	HEX (*Influenza virus* B)	ROX (*Influenza virus* A)	
C–	RNA extraction	**pos**	neg	neg	OK
NCA	Amplification	neg	nd	nd	OK
$C+_A$	Amplification	neg	neg	**pos**	OK
$C+_B$	Amplification	neg	**pos**	neg	OK
CS+	Amplification	**pos**	neg	neg	OK

10. TROUBLESHOOTING

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

- The amplification and detection for samples with the **nd** result (except NCA) should be repeated. If the same result is obtained, repeat analysis of such samples starting from the extraction step. Result **nd** for NCA is normal.
- The amplification and detection for samples with the **eq** result should be repeated. If the same result is obtained, the sample is considered to be **positive**.
- The absence of positive signal for $C+_A$ and/or $C+_B$ and/or CS+ samples indicates incorrect programming of the temperature profile of the thermocycler, incorrect configuration of the PCR reaction, or noncompliance of storage conditions of the kit components with the manufacturer's instruction, or expiration of the reagent kit. Check programming of the thermocycler (see 8.3.2.), storage conditions, and the expiration date of reagents and repeat PCR reaction once again for all samples.

- Positive signal in negative controls (except for C– in FAM channel) indicates contamination of reagents or samples. In this case, the results of analysis should be considered as invalid. Repeat analysis and take measures to detect and elimination the source of contamination.

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/B-FEP 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-FEP** PCR kit are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® *Influenza virus* A/B-FEP** PCR kit are stable until the expiry date stated 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 *Influenza virus* A/B is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

Analytical sensitivity of **AmpliSens® *Influenza virus* A/B-FEP** PCR kit is not less than $1x10^4$ copies per 1 ml of sample (copies/ml).

The claimed analytical features of **AmpliSens® *Influenza virus* A/B-FEP** 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-FEP** PCR kit is ensured by selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

The clinical specificity of **AmpliSens® *Influenza virus* A/B-FEP** PCR kit was confirmed in laboratory clinical trials.

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 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/B-FEP** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number	Σ	Sufficient for
LOT	Batch code		Expiration Date
IVD	*In vitro* diagnostic medical device		Consult instructions for use
VER	Version		Keep away from sunlight
	Temperature limitation	**NCA**	Negative control of amplification
	Manufacturer	**C–**	Negative control of extraction
	Date of manufacture	**$C+_A$, $C+_B$, CS+**	Positive controls of amplification
EC REP	Authorised representative in the European Community	**IC**	Internal control
	Caution		

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
09.12.10	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/B is to be kept away from light was added
	Key to Symbols Used	The explanation of symbols was corrected
	Footer	Reference numbers were changed from V36-R0,2-FEP-CE; V36-R0,5-FEP-CE to V36-50-R0,2-FEP-CE; V36-50-R0,5-FEP-CE
23.06.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”