

# AmpliSens® TBE-FRT PCR kit



For Professional Use Only

## Instruction Manual

### KEY TO SYMBOLS USED

	Catalogue number		Caution
	Batch code		Sufficient for
	<i>In vitro</i> diagnostic medical device		Use-by Date
	Version		Consult instructions for use
	Temperature limit		Keep away from sunlight
	Manufacturer		Negative control of amplification
	Date of manufacture		Negative control of extraction
	Authorized representative in the European Community		Positive control of Amplification
			Internal control

### 1. INTENDED USE

AmpliSens® TBE-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *tick-borne encephalitis virus* RNA in the biological material (blood plasma and serum; leucocytic fraction of blood; cerebrospinal fluid; autopsy material of human and animal (brain tissue); ticks) using real-time hybridization-fluorescence detection of amplified products.

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

### 2. PRINCIPLE OF PCR DETECTION

*Tick-borne encephalitis virus* detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific primers. In the real-time PCR the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The real-time 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® TBE-FRT PCR kit is a qualitative test that contains the Internal Control (Internal Control STI-87-rec (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® TBE-FRT PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by the separation of nucleotides and Taq-polymerase using chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

The results of amplification are registered in the following fluorescence channels:

Table 1

Channel for fluorophore	FAM	JOE
cDNA-target	TBE cDNA	cDNA Internal Control STI-87-rec (IC)
Target gene	C gene	Artificially synthesized sequence

### 3. CONTENT

AmpliSens® TBE-FRT PCR kit is produced in 1 form:

variant FRT-100 F R-V52(RG)-CE.

Variant FRT-100 F includes:

Reagent	Description	Volume, ml	Quantity
RT-PCR-mix-1-FEP/FRT TBE	clear liquid from colorless to light lilac colour	0.6	2 tubes
RT-PCR-mix-2-FEP/FRT	colorless clear liquid	0.3	2 tubes
RT-G-mix-2	colorless clear liquid	0.015	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	2 tubes
TM-Revertase (MMIv)	colorless clear liquid	0.015	2 tubes
Positive Control cDNA TBE (C+TBE)	colorless clear liquid	0.2	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube
Positive Control TBE-rec	colorless clear liquid	0.1	2 tubes
Internal Control STI-87-rec (IC)*	colorless clear liquid	0.12	10 tubes

\* add 10 µl of Internal Control during the RNA extraction procedure directly to the sample/lysis mixture (see RIBO-prep K2-9-Et-100-CE protocols).

Variant FRT-100 F is intended for 120 reactions, including controls.

### 4. ADDITIONAL REQUIREMENTS

- RNA extraction kit.
- Disposable powder-free gloves and a laboratory coat.
- 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.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia).
- Disposable polypropylene microtubes for PCR with 0.5 (0.2) ml capacity (for example, Axygen, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 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 a 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 disposable protective gloves and laboratory cloths, and 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 the PCR kit if the internal packaging was damaged or its appearance was changed.
- Do not use the PCR kit if the transportation and storage conditions according to the Instruction Manual were not observed.
- 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 in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid samples and reagents contact with the skin, eyes, and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
- Safety Data Sheets (SDS) are available on request.
- The PCR kit is intended for single use for PCR analysis of specified number of samples (see the section "Content").
- The PCR kit is ready for use in accordance with the Instruction Manual. Use the PCR kit strictly for intended purpose.
- 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 manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® TBE-FRT PCR kit is intended for analysis of the RNA extracted with RNA extraction kits from the biological material (blood plasma, blood serum; leucocytic fraction of blood; cerebrospinal fluid (CSF), internal organs of animals and autopsy material, tick suspension).

Store the clinical material before the analysis for 1 day at the temperature 2-8 °C, for 1 week at the temperature below minus 16 °C, then - at the temperature below minus 68 °C. Store brain tissue for 1 week at the temperature below minus 16 °C, then - at the temperature below minus 68 °C. Store ticks for 1 month (alive) or for 1 week at the temperature below minus 16 °C, then - at the temperature below minus 68 °C.

— **blood plasma, blood serum; leucocytic fraction of blood; cerebrospinal fluid (CSF)**

Collect the whole blood in the morning after overnight fasting in a tube with 6 % EDTA solution in the ratio 1:20. Overturn the closed tube several times. To collect plasma centrifuge the tube at 3,000 g for 10 minutes. Then remove plasma for the test.

To obtain leucocytic fraction of blood transfer 1.5 ml of the blood with EDTA in a Eppendorf tube and centrifuge at 800 g for 10 minutes. Then transfer top plasma layer containing leucocytes (500-600 µl) in a second Eppendorf tube and centrifuge at 11,000 g for 10 minutes. Remove and discard the supernatant. Use the cell pellet and 100 µl of supernatant above the pellet for RNA extraction.

Pretreatment is not required for blood serum and CSF.

— **animal internal organs and autopsy material**

Homogenize internal organs of animals and autopsy material using a porcelain mortar and pestle and prepare 10 % suspension with sterile saline solution or phosphate buffer. Use 50 µl of the suspension for RNA extraction.

— **ticks suspension**

If ticks pools are used for the analysis, the number of ticks in one pool should not exceed 10. For *Dermacentor* genus it is preferable to analyze individual ticks. Place the ticks into the Eppendorf tubes, add 500 µl of 96 % ethanol and shake on vortex. Centrifuge the tubes with ticks at 5,000 rpm for 3-5 sec then remove fluid by vacuum aspirator. To the tubes with ticks add 500 µl of 0.15 M sodium chloride solution, vortex, and spin at 5,000 rpm for 5 sec. Remove fluid by vacuum aspirator. To make ticks suspension use sterile porcelain mortar and a pestle. Homogenize ticks in 300 µl (if the sample consists of 1 tick) or 1 ml (if a tick pool is analyzed) of 0.15 M sodium chloride solution. Add the solution by small portion while homogenizing. Centrifuge prepared suspension at 5,000 rpm for 2 min and use 100 µl of supernatant for RNA extraction.

## 7. WORKING CONDITIONS

AmpliSens® TBE-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-prep **REF** K2-9-Et-50-CE or **REF** K2-9-Et-100-CE.

RNA extraction of each test sample is carried out in the presence of **Internal Control STI-87-rec (IC)**.

In the extraction procedure it is necessary to carry out the control reaction as follows:

- C-** – Add **10 µl of Internal Control STI-87-rec (IC)** and **300 µl of Solution for Lysis** to the tube labelled C- (Negative Control of Extraction).
- PCE** – Add **10 µl of Positive Control TBE-rec** to the tube labeled **PCE (Positive Control of Extraction)**

Extract the RNA according to the manufacturer's protocol taking into account next additions and improvements:

- Add to the tubes with **Solution for Lysis** and **Internal Control STI-87-rec (IC)**:

- NOTE:**
- **100 µl of prepared samples;**
  - **200 µl of the CSF sample** or
    - **50 µl of the internal organ suspension.**
  - If extracting from cells pellets: add **300 µl of Solution for Lysis** and **10 µl of IC STI-87-rec** directly to the tubes with the samples.

### 8.2. Preparing the reverse transcription and PCR

#### 8.2.1 Preparing tubes for PCR

##### Variant FRT

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

1. Prepare the required number of the PCR tubes.
2. Prepare the reaction mixture for required number of reactions. To do this, mix in a clean tube **RT-PCR-mix-1-FEP/FRT TBE**, **RT-PCR-mix-2-FEP/FRT**, **polymerase (TaqF)**, **RT-G-mix-2**, **TM-Revertase (MMIv)** calculating per **each** reaction:
  - **10 µl RT-PCR-mix-1-FEP/FRT TBE;**
  - **5 µl RT-PCR-mix-2-FEP/FRT;**
  - **0.5 µl polymerase (TaqF);**
  - **0.25 µl TM-Revertase (MMIv);**
  - **0.25 µl RT-G-mix-2;**

**NOTE:** Note that each run includes at least four controls: Positive and Negative Controls of extraction (PCE, C-) as well as Positive and Negative Controls of amplification (C+, NCA).

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3. Transfer **15 µl** of prepared reaction mix per each tube.

**NOTE:** Do not store the prepared mixture

4. Using tips with aerosol filter add **10 µl of RNA samples** obtained at the RNA extraction stage. Carefully mix by pipetting.

5. Carry out control amplification reactions:

- NCA** – Add **10 µl of DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).
- C+TBE** – Add **10 µl of Positive Control cDNA TBE** to the tube labeled C+TBE (Positive Control of Amplification).

**NOTE:** Perform the amplification reaction immediately after cDNA samples and controls are added to the reaction mixture.

#### 8.2.2. Amplification

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

Table 2

Amplification program			
Step	Temperature, °C	Time	Cycles
Hold	50	30 min	1
Hold 2	95	15 min	1
Cycling	95	10 sec	5
	65	45 sec	
	72	15 sec	
Cycling 2	95	10 sec	45
	60	45 sec Fluorescence acquiring	
	72	15 sec	

Fluorescent signal is detected in the channels for the FAM and JOE fluorophores.

2. Adjust the fluorescence channel sensitivity according to the Guidelines [2].

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

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in two channels:

- The signal of the TBE cDNA amplification product is detected in the channel for the FAM fluorophore.
- The signal of the IC cDNA amplification product is detected in the channel for the JOE fluorophore.

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a Ct value of the cDNA sample in the corresponding column of the results grid.

Principle of interpretation is the following:

- TBE cDNA is **detected** if the Ct value determined in the results grid in the channel for the FAM fluorophore is less than the boundary Ct value. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
- TBE cDNA is **not detected** in a sample if the Ct value is not determined (absent) in the channels for FAM fluorophore, whereas the Ct value determined in the channel for the JOE fluorophore is less than the boundary Ct value.
- The result of is **valid** if the Ct value determined in the channel for the JOE fluorophore is less than the boundary Ct value.
- The result is **invalid** if the Ct value is not determined (absent) in the channel for FAM fluorophores, whereas the Ct value in the channel for the JOE fluorophore is greater than the specified boundary Ct value. In such cases, the PCR analysis should be repeated starting from the RNA extraction stage.

**NOTE:** Boundary Ct values are specified in Guidelines [2]

The result of the analysis is considered reliable only if the results obtained for the Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Table 3).

Table 3

Results for controls			
Control	Stage for control	Ct value in the channel for fluorophore	
		FAM	JOE
C-	RNA extraction	Absent	< boundary value
PCE	RNA extraction	< boundary value	< boundary value
NCA	PCR	Absent	Absent
C+TBE	PCR	< boundary value	Absent

## 10. TROUBLESHOOTING

Results of analysis are not being registered in the following cases:

1. If the Ct value is determined for the Negative Control of Extraction (C-) in the channel for the FAM fluorophore and/or for the Negative control of amplification (NCA) in the channels for FAM, JOE fluorophores, the contamination of reagents or test samples is probable. In this case the results of the analysis for all samples are considered invalid. The PCR analysis should be repeated for all samples. Measures for detecting and elimination of contamination source must be taken.
2. If the Ct value is not determined (absent) for the Negative control of Extraction (C-) in the channel for the JOE fluorophore and/or for the Positive control of Extraction (PCE) in the channels for FAM, JOE fluorophores, the results of the analysis for all samples are considered invalid. It is necessary to repeat the analysis of all samples beginning with the RNA extraction stage.
3. If the Ct value is not determined (absent) for the Positive control of Amplification (C+) in the channel for the FAM fluorophore, the results of the analysis for all samples are considered invalid. It is necessary to repeat the analysis for all samples beginning with the RT-PCR stage.

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

## 11. TRANSPORTATION

AmpliSens® TBE-FRT PCR kit should be transported at 2–8 °C for no longer than 5 days.

## 12. STABILITY AND STORAGE

All components of the **AmpliSens® TBE-FRT PCR kit** (except for RT-G-mix-2, RT-PCR-mix-1-FEP/FRT TBE, RT-PCR-mix-2-FEP/FRT, polymerase (TaqF), and TM-Revertase (MMIv)) are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® TBE-FRT 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.

**NOTE:** RT-G-mix-2, RT-PCR-mix-1-FEP/FRT TBE, RT-PCR-mix-2-FEP/FRT, polymerase (TaqF), and TM-Revertase (MMIv) are to be stored at the temperature from minus 24 to minus 16 °C.

**NOTE:** RT-PCR-mix-1-FEP/FRT TBE is to be kept away from light.

## 13. SPECIFICATIONS

### 13.1. Sensitivity

Analytical sensitivity of **AmpliSens® TBE-FRT PCR kit** is no less than 1x10<sup>3</sup> copies per 1 ml of sample (copies/ml).

The claimed analytical features of **AmpliSens® TBE-FRT PCR kit** are guaranteed only when additional reagents kit, RIBO-prep, (manufactured by Federal Budget Institute of Science "Central Research Institute for Epidemiology") is used.

### 13.2. Specificity

The analytical specificity of **AmpliSens® TBE-FRT PCR kit** is ensured by the 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® TBE-FRT PCR kit** was confirmed in laboratory clinical trials.

## 14. REFERENCES

- 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.
- Guidelines to the **AmpliSens® TBE-FRT** PCR kit for qualitative detection of *tick-borne encephalitis virus* RNA in the biological material by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection developed by Federal Budget Institute of Science "Central Research Institute for Epidemiology".

## 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® TBE-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
26.12.10 RT	Cover page	The phrase "For Professional Use Only" was added
	Intended use	The phrase "The results of PCR analysis are taken into account in complex diagnostics of disease" 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 open reagents was added
Information that RT-PCR-mix-1-FEP/FRT TBE is to be kept away from light was added		
Key to Symbols Used	The explanation of symbols was corrected	
29.06.11 LA	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science "Central Research Institute for Epidemiology"
17.01.17 PM	Through the text	Corrections according to the template
	8.1 RNA extraction	The sections were rewritten
	9. Data analysis	
	10. Troubleshooting	
14. References	The reference to the Guidelines was added	
13.03.19 EM	3. Content	The colour of the reagent was specified
30.04.20 MA	Through the text	The text formatting was changed
	Footer	The phrase "Not for use in the Russian Federation" was added
	2. Principle of PCR detection	The table with targets was added
11.03.21 MM	—	The name, address and contact information for Authorized representative in the European Community was changed

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