



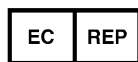
For *in Vitro* Diagnostic Use

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AmpliSens® TBE-FRT PCR kit

Instruction Manual



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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), by means of real-time RT-PCR with hybridization-fluorescence detection.

2. PRINCIPLE OF PCR DETECTION.

Tick-borne encephalitis virus detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special 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 real-time PCR monitoring of the 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 and contains the Internal Control (IC) which must be used in the isolation procedure in order to control the isolation process of each individual sample and to identify possible reaction inhibition. **AmpliSens® TBE-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 chemically modified polymerase (TaqF). Chemically modified polymerase (TaqF) activates by heating at 95°C for 15 min.


3. CONTENT.

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

AmpliSens® TBE-FRT PCR kit variant FRT-100 F (for use with RG)  R-V52(RG)-CE

AmpliSens® TBE-FRT PCR kit includes:

Reagent	Description	Volume (ml)	Amount
RT-PCR-mix-1-FEP/FRT TBE	colorless, clear liquid	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 (MMLV)	colorless, clear liquid	0.015	2 tubes
Positive Control cDNA TBE (C+)	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 STI-87-rec during the RNA isolation procedure directly to the sample/lysis mixture (see “RIBO-prep”  K2-9-Et-100-CE protocols).

AmpliSens® TBE-FRT FRT PCR kit is intended for 120 reactions, including controls.

4. ADDITIONAL REQUIREMENTS.

- RNA isolation kit.
- Disposable powder-free gloves.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers up to 200 µl.
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2 ml reaction tubes.
- PCR box.
- Rotor-Gene™ 3000 or Rotor-Gene™ 6000 Instrument (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 with temperature below minus 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 filters and use new tip for every procedure.
- Store extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats and eye protection when handling specimens and reagents. 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.
- Specimens 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 other suitable disinfectant.
- Avoid sample or reagent contact with the skin, eyes and mucose membranes. If any of 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 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 of biological material samples for PCR-analysis, transportation and storage are described in manufacturer’s handbook [1]. It is recommended that this handbook is read before starting of the work.

AmpliSens® TBE-FRT PCR kit is intended for the analysis of RNA extracted with RNA isolation kits from:

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

Take whole blood specimen in the morning after overnight fasting in a tube with 6 % EDTA solution in the ratio 1:20. Invert 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 leukocytic fraction of blood transfer 1.5 ml of the blood with EDTA in a tube (like Eppendorf tube) and centrifuge at 800 g for 10 minutes. Then transfer top plasma layer containing leucocytes (500-600 µl) in a second tube (like 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.

Blood serum and CSF do not need preprocessing.

— internal organs of animals and autopsy material

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

— ticks suspension

If ticks pool is examined, the number of ticks per pool should not exceed 10. For *Dermacentor* genus an examination of a single tick is preferable. Place ticks into the tubes (like Eppendorf), add 500 µl of 96% ethanol and shake on vortex. Centrifuge the tubes with ticks at 5,000 r/min 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 the pool consists of several ticks) 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. PROTOCOL.

7.1. RNA Isolation

It's recommended using of the following nucleic acid extraction kits:

- RIBO-prep **REF** K2-9-Et-50-CE



Carry out the RNA isolation according to the manufacturer's instructions



Volume of the samples for RNA extraction:

- tissue homogenates – 50 µl
- CSF – 200 µl
- Positive Control of extraction (PC TBE-rec) – 10 µl



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



The tube of Negative Control of extraction should only include 300 µl of Solution for Lysis and 10 µl of IC STI-87-rec only.

7.2. Preparing the PCR.

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

7.2.1 Preparing tubes for PCR.

Variant FRT

1. Prepare the required number of the PCR tubes.
2. Prepare the reaction mix 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;

Take into account that each run includes at least for control points: Positive and Negative Controls of extraction (PCE, C-) as well as Positive and Negative Controls of amplification (C+, NCA).

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



Do not store prepared mix!

4. Using tips with aerosol barrier add 10 µl of RNA samples obtained from clinical or control samples at the stage of RNA extraction into prepared tubes. 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+ -Add 10 µl of Positive Control cDNA TBE to the tube labeled C+ (Positive Control of Amplification).

Amplification should immediately follow by compounding of the reaction mix with RNA-samples and controls.

7.2.2. Amplification

RG

1. Program the Rotor-Gene™ according to manufacturer's manual and Appendix 1.
2. Create a temperature profile on your Rotor-Gene™ instrument as follows:

Step	Temperature, °C	Time	Fluorescence detection	Repeats
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	FAM/Green, JOE/Yellow	
	72	15 sec	-	

3. Make the adjustment of the fluorescence channel sensitivity according to Appendix 1.

8. DATA ANALYSIS.

Internal Control is detected in the JOE/Yellow fluorescence channel, TBE RNA is detected in the FAM/Green fluorescence channel.

See **Appendix 1**, for data analysis settings for Rotor-Gene™ 3000/6000.

Results interpretation

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

Results for controls

Control	Stage for control	Ct in channel		Interpretation
		FAM	JOE	
C-	RNA isolation	Neg	Pos (< X*)	OK
PCE	RNA isolation	Pos (< F*)	Pos (< J*)	OK
NCA	Amplification	Neg	Neg	OK
C+	Amplification	Pos (< Y*)	Neg	OK

*For F, J, X, Y, values see Appendix 1.

- The sample is considered **positive** if its Ct value on FAM/Green channel is less than A**.
If Ct value if FAM/Green channel is more than A, the result is considered equivocal. This RNA-sample should be re-examined in two runs.
If Ct value in FAM/Green channel is more than A, while Ct value in JOE/Yellow channel is absent, the result is considered invalid (see paragraph 3 below).
- The sample is considered **negative** if Ct value on FAM/Green channel exceeds A or is absent, while Ct value in JOE/Yellow channel does not exceed B**.
- The result of a sample is considered **valid** if its Ct value in JOE/Yellow channel does not exceed B.
If Ct value in JOE/Yellow channel is more than B, while Ct value in FAM/Green channel is absent, the result is considered **invalid**. It is necessary to repeat the analysis for this sample starting from the extraction stage.

*For A, B values see Appendix 1.

Results are accepted as relevant if both positive and negative controls of amplification along with negative and positive controls of extraction are passed.

9. TROUBLESHOOTING.

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

- Presence of any Ct value for negative control of extraction (C-) in FAM/Green channel and/or for negative control of amplification (NCA) in FAM/Green, JOE/Yellow channels indicates the contamination of reagents or samples. In this case results of the analysis for all samples are considered invalid. It is necessary to repeat the analysis, and also to take measures to detect and eliminate the source of contamination.
- If Ct value for negative control of extraction (C-) in JOE/Yellow channel and/or for positive control of extraction (PCE) in FAM/Green, JOE/Yellow channels is absent, results of the analysis for all samples are considered invalid. It is necessary to repeat the analysis of all samples starting from the extraction stage.
- If Ct value for positive control of amplification (C+) in FAM/Green channel is absent, results of the analysis for all samples are considered invalid. It is necessary to repeat the RT-PCR for all samples.

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

10. 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 (MMLv)) are to be stored between 2 and 8 °C, when not in use. They also must be stable until the expiry date stated on the label.



RT-G-mix-2, RT-PCR-mix-1-FEP/FRT TBE, RT-PCR-mix-2-FEP/FRT, polymerase (TaqF) and TM-Revertase (MMLv) should be stored at not more than minus 16 °C

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens® TBE-FRT** PCR kit is not less than 1×10^3 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 State Institution of Science Central Research Institute of Epidemiology) is used.

11.2. Specificity.

Specificity of **AmpliSens® TBE-FRT** PCR kit is assured by selection of specific primers and probes, as well as the selection of strict 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® TBE-FRT** PCR kit was confirmed in laboratory clinical trials.

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 compliance with Federal State Institution of Science "Central Research Institute of 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.

14. EXPLANATION OF SYMBOLS.



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