ECOLI

eSens

CE

IVD

For Professional Use Only

eSens TBEV QL PCR Kit

REF ES3702A

Instructions for Use

1 INTENDED USE

eSens TBEV QL 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.

eSens TBEV QL 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.

eSens TBEV QL 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

eSens TBEV QL PCR Kit (ES3702A) 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 (MMlv)	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

eSens TBEV QL PCR Kit 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 (e.g Rotor-Gene Q or equivalent).
- 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

eSens TBEV QL PCR Kit is intended for analysis of the RNA extracted with RNA extraction kits from the biological material (blood plasma, blood serum; leukocytic 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; leukocytic 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 leukocytic 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

eSens TBEV QL PCR Kit should be used at 18–25 °C.

8 PROTOCOL

8.1 RNA extraction

Any commercial nucleic acid extraction kit, if IVD-CE validated for the indicated specimen types, could be used.

Ecoli Dx, s.r.o. recommends:

- For the manual extraction

RIBO-prep (K2-9-Et-100-CE).

- For the automatic extraction

- **ePure Viral Nucleic acid Extraction Kit** (E2003)

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)

8.2 Preparing the reverse transcription and PCR

8.2.1 Preparing tubes for PCR

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 (MMlv)** 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 (MMlv);
   - 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).

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
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.

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

Table 4

Boundary *Ct* values

Sample	**Channel**	***Ct* value**
C-	JOE/Yellow	28
PCE	FAM/Green	37
	JOE/Yellow	28
$C+_{TBE}$	FAM/Green	29
Test samples	FAM/Green	37
	JOE/Yellow	28

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-RCR stage.

10 TRANSPORTATION

eSens TBEV QL PCR Kit should be transported at 2–8 °C for no longer than 5 days.

11 STABILITY AND STORAGE

All components of the **eSens TBEV QL 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 at 2–8 °C when not in use. All components of the **eSens TBEV QL 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 (MMlv) are to be stored at the temperature from minus 24 to minus 16 °C.
- RT-PCR-mix-1-FEP/FRT TBE is to be kept away from light.

12 SPECIFICATIONS

12.1 Sensitivity

Analytical sensitivity of **eSens TBEV QL PCR Kit** is no less than $1x10^3$ copies per 1 ml of sample (copies/ml).

12.2 Specificity

The analytical specificity of **eSens TBEV QL 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 **eSens TBEV QL PCR Kit** was confirmed in laboratory clinical trials.

13 QUALITY CONTROL

The production process, including batch release, is carried out in accordance with an established quality management system certified according to ISO 13485.

14 KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Contains sufficient for <n> tests
IVD	*In vitro diagnostic* medical device		Use-by Date
VER	Version		Consult instructions for use
	Temperature limit		Keep away from sunlight
	Manufacturer	NCA	Negative control of amplification
	Date of manufacture	C-	Negative control of extraction
EC REP	Authorized representative in the European Community	$C+_{TBE}$	Positive control of amplification
		IC	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01_04/2022		

Ecoli Dx, s.r.o. , Purkyňova 74/2

110 00 Praha 1, Česká republika
Tel: +420 325 209 912

Mobil: +420 739 802 523