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For Professional Use Only

eSens Borrelia miyamotoi QL PCR kit

REF ES3701B

Instructions for Use

1 INTENDED USE

eSens Borrelia miyamotoi QL PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Borrelia miyamotoi* DNA in the biological material (blood, tissue (autopsy, biopsy) material, cerebrospinal fluid, ticks) using real-time hybridization-fluorescence detection of amplified products.

Indications and contra-indications for use of the reagent kit

The reagent kit is used for the analysis of biological material, taken from the persons suspected of ixodic tick-borne borreliosis without distinction of form and presence of manifestation, and ticks.

There are no contra-indications with the exception of cases when the material cannot be taken for medical reasons.

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

2 PRINCIPLE OF PCR DETECTION

Borrelia miyamotoi detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *Borrelia miyamotoi* 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 Borrelia miyamotoi QL PCR kit is a qualitative test that contains the Internal Control (Internal Control-FL (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 Borrelia miyamotoi QL PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by using chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

eSens Borrelia miyamotoi QL PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and deoxyuridine triphosphate (dUTP). The enzyme UDG recognizes and catalyzes the destruction of the DNA containing deoxyuridine, but has no effect on DNA containing deoxythymidine. Deoxyuridine is absent in the authentic DNA, but is always present in amplicons, because dUTP is a part of dNTP mixture in the reagents for the amplification. Due to the deoxyuridine containing contaminating amplicons are sensitive to the destruction by UDG before the DNA-target amplification. So the amplicons cannot be amplified.

The enzyme UDG is thermolabile. It is inactivated by heating at temperature above 50 °C. Therefore, UDG does not destroy the target amplicons which are accumulated during PCR.

At the amplification stage 2 reactions are carried out in one tube simultaneously: amplification of *Borrelia miyamotoi* DNA as well as amplification of Internal Control-FL(IC) DNA. The results of amplification of *Borrelia miyamotoi* DNA and Internal Control-FL (IC) DNA are registered in 2 different fluorescence channels.

Table 1

Channel for fluorophore	FAM	ROX
DNA-target	IC DNA	*Borrelia miyamotoi* DNA
Target gene	Artificial nucleotide sequence	glpQ gene

3 CONTENT

eSens Borrelia miyamotoi QL PCR kit (ES3701B) includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-FL *Borrelia miyamotoi*	clear liquid from colorless to light lilac colour	0.6	1 tube
PCR-buffer-H	colorless clear liquid	0.3	1 tube
C+ *Borrelia miyamotoi*	colorless clear liquid	0.2	1 tube
TE-buffer	colorless clear liquid	0.2	1 tube
Negative Control (C–)*	colorless clear liquid	1.2	1 tube
Internal Control-FL (IC)**	colorless clear liquid	0.5	1 tube

***** must be used in the extraction procedure as Negative Control of Extraction.

****** add **10 µl** of **Internal Control-FL (IC)** during the DNA extraction procedure directly to the sample/lysis mixture.

eSens Borrelia miyamotoi QL PCR kit is intended for 55 reactions (including controls).

4 ADDITIONAL REQUIREMENTS

- 0.9 % of sodium chloride (sterile saline solution) or phosphate buffered saline (PBS) (137 mM sodium chloride; 2,7 mM potassium chloride; 10 mM sodium monophosphate; 2 mM potassium diphosphate; pH=7,5±0,2).
- 96 % ethanol for ticks pretreatment.
- Glycerin for the storage of pretreated ticks.
- Vacuum blood collection system.
- Puncture needles.
- Sterile plastic container (50-60 ml) for sampling, storage and transportation of biological samples.
- Flocked swab for collection, transportation and storage of biological samples.
- Sterile tools (individual for each sample) for homogenization (porcelain mortar and mallet) or homogenizer for pretreatment of tissue material and ticks.
- Vacuum aspirator with flask for removing supernatant.
- DNA extraction kit.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters (up to 100, 200, 1,000 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- PCR box.
- Real-time instruments (for example, Rotor-Gene Q (QIAGEN, Germany), CFX 96 Touch, CFX 96 Opus (Bio-Rad, USA), QuantStudio 5 (Thermo Fisher Scientific), or equivalent).
- Disposable polypropylene PCR tubes:
  a) tightly closed 1.5 and 2-ml tubes for sampling.
  b) screwed or tightly closed 1.5 and 2-ml tubes for pretreatment.
  c) screwed or tightly closed 1.5-ml tubes for reaction mixture preparation.
  d) thin-walled 0.2-ml PCR tubes with optical transparent domed or flat caps or strips of eight 0.2-ml tubes with optical transparent caps if a plate-type instrument is used;
  e) thin-walled 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used.
- 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) away from all other reagents and add it to the reaction mix in a distantly separated facility. Store and handle amplicons away from all other reagents.
- 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 inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- While observing the conditions of transportation, operation and storage, there are no risks of explosion and ignition.
- 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 Borrelia miyamotoi QL PCR kit is intended for analysis of the DNA extracted with DNA extraction kits from the biological material (blood, tissue (autopsy, biopsy) material, cerebrospinal fluid, ticks.

Sampling

6.1 Blood.

For the method sensitivity increasing, the bacterial pellet of blood is analyzed. To obtain the pellet, blood should be taken after overnight fasting or in 3 hour after eating by a disposable 0.8-1.1 mm diameter needle into the tube (special vacuum system) with EDTA or sodium citrate solution as anticoagulant. After blood sampling the tube should be smoothly rotated several times for the thoroughly mixing with the anticoagulant. (Otherwise, blood will coagulate and DNA extraction will be impossible!). Place the tube in the rack after rotating.
Blood samples can be stored before the obtaining and preparing the bacterial pellet:

- at the temperature from 20 to 25 °C – for 2 hour;
- at the temperature from 2 to 8 °C – for 12 hour;

Freezing of whole blood samples is unallowable!
The samples are to be prepared no later than the specified time.

6.2 Tissue (autopsy, biopsy) material.

The material is taken from the area of probable location of infection agent, from the lesional tissue or the area surrounding lesional tissue or from the unaltered fragments of organ tissues: brain, heart, lung,

liver, spleen, nephros, by a sterile tool (for example, tweezers) into a sterile plastic 50-ml container with tightly closed cap or 2 ml tube. The tube is to be closed tightly.
The tissue material samples can be stored:

- at room temperature – for 6 hour,
- at the temperature from 2 to 8 °C – for 3 days,
- at the temperature from minus 24 to minus 16 °C – for 1 week,
- at the temperature ≤ –68 °C – for a long time.

6.3 Cerebrospinal fluid.

Cerebrospinal fluid is collected in an amount no less than 1 ml by sterile needle into disposable 2.0-ml tubes.
The cerebrospinal fluid samples can be stored before the PCR analysis:

- at the temperature from 2 to 8 °C – for 1 day,
- at the temperature from minus 24 to minus 16 °C – for 1 week,
- at the temperature ≤ –68 °C – for a long time.

Only one freeze-thawing cycle is required.

6.4 Ticks.

The collected material is sorted into species, sex, places and dates of collection and placed into the dry sterile 2.0-ml tube. Number of ticks in pool for analysis should not exceed 10.

The material samples can be stored after sorting and samples formation:

- at the temperature from minus 24 to minus 16 °C – for 1 month;
- at the temperature not more than minus 68 °C or in the Dewar flask with liquid nitrogen – for a long time.

Only one freeze-thawing cycle is required.
The above mentioned material can be transported at the temperature from 2 to 8 °C for 1 day.

Pretreatment

To obtain the *bacterial pellet* of blood the pretreatment of blood samples is required.
Using a filter tip transfer 1.5 ml of blood with EDTA into the sterile disposable 2.0-ml tube. Centrifuge at 40 g (for example, 800 rpm for the MiniSpin Eppendorf microcentrifuge) for 10 min. Using a new one filter tip transfer 500-600 µl of supernatant (plasma with leucocytes) into sterile disposable 1.5-ml tube (do not take the pellet with erythrocytes). Centrifuge at 10,000 g (for example, 12,000 rpm for the MiniSpin Eppendorf microcentrifuge) for 10 min.
The bacterial pellet samples can be stored before the PCR analysis:

- at the temperature from minus 24 to minus 16 °C – for 1 week,
- at the temperature not more than minus 68 °C – for a long time.

6.5 Tissue (autopsy, biopsy) material.

Tissue (autopsy, biopsy) material is to be pretreated. For DNA extraction take 30-50 mg (µl) of the material and homogenize it by trituration using precooled sterile porcelain mortar and mallet or homogenizer. Prepare 10 % suspension using grinded tissue and precooled 0.9 % sodium chloride solution (sterile saline solution) or phosphate buffer (PBS). For this, add 9 volumes of saline solution or phosphate buffer to 1 volume of grinded tissue. Use 100 µl of obtained suspension for DNA extraction.

The pretreated biopsy material samples can be stored:

- at the temperature from minus 24 to minus 16 °C – for 1 week,
- at the temperature not more than minus 68 °C – for a long time.

6.6 Cerebrospinal fluid.

Cerebrospinal fluid is to be pretreated. Centrifuge 1-1.5 ml of cerebrospinal fluid at 10,000 g (for example, 12,000 rpm for the MiniSpin Eppendorf microcentrifuge) for 10 min. Discard the supernatant into the reservoir for the waste dispose. Use the cells pellet in 100 µl of supernatant for DNA extraction.

The cerebrospinal fluid samples can be stored before the PCR analysis:

- at the temperature from minus 24 to minus 16 °C – for 1 week,
- at the temperature not more than minus 68 °C – for a long time.

The material freeze-thawing of is not allowed without the DNA extraction procedure.

6.7 Ticks.

Ticks are to be pretreated. If pools of ticks are used for the analysis, number of ticks in one pool should not exceed 10. For ixodic tick of all genus (except for *Ixodes*) it is preferable to analyze individual ticks. Place the ticks in 1.5-ml tubes, add **500 µl** of 96 % ethanol. Mix and sediment on vortex. Discard ethanol from each tube by a separate tip without filter using vacuum aspirator. Add **500 µl** of 0.9 % sodium chloride solution (sterile saline solution) or phosphate buffer (PBS). Mix and sediment on vortex. Discard supernatant by a separate tip without filter using vacuum aspirator. Transfer the ticks into the sterile porcelain mortar, add **300 µl** (if the sample consists of 1 *Ixodes* tick), **600 µl** (if the sample consists of ixodic tick of any genus, except for *Ixodes*) and **1 ml** (if a pool of ticks is analyzed) of 0.9 % sodium chloride solution (sterile saline solution) or phosphate buffer (PBS). Homogenize the sample.

Using a separate filter tip transfer the sample into 1.5-ml tube and centrifuge at 2,000 g (for example, 5,000 rpm for the MiniSpin Eppendorf microcentrifuge) for 2 min for the clarification of the sample. Remove **100 µl** of supernatant. Add glycerin into the rest of suspension (10 % from the volume of the rest of suspension). Mix the sample and freeze it at the temperature from minus 24 to minus 16 °C for the subsequent PCR analysis.
The pretreated ticks can be stored before the PCR analysis:

- at the temperature from minus 24 to minus 16 °C – for 1 week,
- at the temperature not more than minus 68 °C or in the Dewar flask with liquid nitrogen – for a long time.

Only one freeze-thawing cycle is required.

Interfering substances and limitations of using test material samples

In order to control the DNA extraction efficiency and possible reaction inhibition the Internal Control (Internal Control-FL (IC)) is used in the PCR kit. The Internal Control is added in each biological sample at the extraction stage. The presence of internal control signal after the amplification testifies the effectiveness of nucleic acid extraction and the absence of PCR inhibitors.
The blood samples, collected in the tubes with heparin as anticoagulant are inapplicable for analysis.

7 WORKING CONDITIONS

eSens Borrelia miyamotoi QL PCR kit should be used at the temperature from 20 to 28 °C and relative humidity from 15 to 75 %.

8 PROTOCOL

8.1 DNA 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 DNA extraction from blood, cerebrospinal fluid, ticks, tissue (autopsy and biopsy) material;

- For the **automatic** extraction

- **ePure Bacterial DNA Extraction Kit** (E2006)

NOTE: Extract the DNA according to the manufacturer's protocol.
The DNA extraction for each sample is carried out in the presence of **Internal Control-F (IC)**.

8.2 Preparing PCR

8.2.1 Preparing tubes for PCR

The type of tubes depends on the PCR instrument used for analysis. Use disposable filter tips for adding reagents, DNA and control samples into tubes.

The total reaction volume is **25 µl**, the volume of the DNA sample is **10 µl**.

1. Calculate the required quantity of each reagent for reaction mixture preparation. For one reaction:

   10 µl of **PCR-mix-FL *Borrelia miyamotoi*,**
   5 µl of **PCR-buffer-H**.

Prepare the reaction mixture for the total number of test and control samples plus one extra reaction. See numbers of control samples in item 7.

NOTE: Prepare the reaction mixture just before use.

2. Thaw the tube with PCR-mix-FL *Borrelia miyamotoi*. Thoroughly vortex all the reagents of the PCR kit and sediment the drops by vortex.
3. In a new tube prepare the reaction mixture. Mix the required quantities of PCR-mix-FL *Borrelia miyamotoi* and PCR-buffer-H. Sediment the drops by vortex.
4. Take the required number of the tubes or strips taking into account the number of test samples and control samples.
5. Transfer 15 µl of the prepared reaction mixture to each tube. Discard the unused reaction mixture.
6. Add 10 µl of DNA samples extracted from test samples at the DNA extraction stage using tips with filter.

NOTE: Mix the tubes thoroughly by pipetting avoiding foaming.

7. Carry out the control reactions:

NCA	–	Add **10 µl** of **TE-buffer** to the tube labeled **NCA** (Negative Control of Amplification).
C+	–	Add **10 µl** of **C+ *Borrelia miyamotoi*** to the tube labeled **C+** (Positive Control of Amplification).
C–	–	Add **10 µl** of **the sample extracted from the C– sample** to the tube labeled **C–** (Negative Control of Extraction).

NOTE: Mix the tubes thoroughly by pipetting avoiding foaming.

NOTE: Carry out the PCR just after the mix of reaction mixture and DNA-samples and controls.

8.2.2 Amplification

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

* It is preferable to use the amplification program in Table 3, if there is no need to use the unified amplification program.

Table 2

eSens unified amplification program

	Rotor-type Instruments (e.g Rotor-Gene Q or equivalent)		**Plate-type Instruments** (e.g CFX 96 Touch, CFX 96 Opus, QuantStudio 5 or equivalent)	
Step	**Temperature, °C**	**Time**	**Fluorescent signal detection**	**Cycles**
1	**50**	**15 min**	**–**	**1**
2	**95**	**15 min**	**–**	**1**
3	**95**	**10 s**	**–**	**45**
	60	**20 s**	**FAM, ROX**	

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

NOTE: Any combination of the tests (including tests with reverse transcription and amplification) can be performed in one instrument simultaneously with the use of the unified amplification program. If several tests in "multiprime" format are carried out simultaneously, the detection is enabled in other used channels except for the specified ones. If in one instrument only the tests for the DNA detection are carried out simultaneously, the first step of reverse transcription (50 °C – 15 min) can be omitted for time saving, but the iteration at 60 °C should be increased up to 30 s in the third step.

Table 3

eSens unified amplification program

	Rotor-type Instruments (e.g Rotor-Gene Q or equivalent)		Plate-type Instruments (e.g CFX 96 Touch, CFX 96 Opus, QuantStudio 5 or equivalent)	
Step	**Temperature, °C**	**Time**	**Fluorescent signal detection**	**Cycles**
1	**95**	**15 min**	–	**1**
2	**95**	**10 s**	–	**45**
	60	**30 s**	**FAM, ROX**	

2. Adjust the fluorescence channel sensitivity.
3. Insert tubes into the reaction module of the device.

NOTE: It is recommended to sediment drops from walls of tubes by short centrifugation (1–3 s) before placing them into the instrument.
Insert empty tubes at the edges of reaction module in case of incomplete filling of plate-type instrument.

4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

8.3 Instrument Settings

Test settings for rotor-type instruments

Channel	Calibrate/Gain Optimisation	Threshold	Dynamic tube	Slope Correct	More Settings/ Outlier Removal
FAM/Green	from 5FI to 10FI	5 %	on	0.03	5
ROX/Orange	from 5FI to 10FI	5 %	on	0.03	5

Note: If the fluorescence curves in the FAM/Green and ROX/Orange channels do not correspond to the exponential growth, then ***NTC threshold value*** can be increased up to **10 %**.

Test settings for plate-type instruments

Note: Set the heating/cooling rate ***Ramp Rate* 2,5 °C/s** by clicking the ***Step Options*** button for each step of cycling.

Channel	Threshold fluorescence
FAM	Set the threshold line at the level corresponding to 10% of maximum fluorescence level obtained for C+ sample at the last amplification cycle.
ROX	Set the threshold line at the level corresponding to 5.% of maximum fluorescence level obtained for C+ sample at the last amplification cycle.

Note: The fluorescence curve of C+ sample is to cross the threshold line at the section of exponential growth passing into linear growth.

9 DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in 2 channels:

Table 4

Channel for the fluorophore	FAM	ROX
Signal registration, indicating the amplification product accumulation	Internal Control-FL (IC) DNA	*Borrelia miyamotoi* DNA

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

Principle of interpretation is the following:

Table 5

Results interpretation

***Ct* value in the channel for the fluorophore**		**Result**
FAM	**ROX**	
< boundary value	absent	*Borrelia miyamotoi* DNA **is not detected**
determined or absent	< boundary value	*Borrelia miyamotoi* DNA **is detected**
absent or > boundary value	absent or > boundary value	**Invalid** result*
< boundary value	> boundary value	**Equivocal** result**

* In case of invalid result, the PCR analysis should be repeated for the corresponding test sample starting from the DNA extraction stage.

** In case of equivocal result, the PCR analysis should be repeated for the corresponding test sample starting from the DNA extraction stage. If the same result is obtained, the sample is considered positive. If the negative result is obtained in the second run, the sample is considered equivocal and re-sampling of the material for analysis is recommended.

The result of the PCR analysis is considered reliable only if the results obtained for the controls of amplification and extraction are correct (see Table 6 and Table 7).

Table 6

Results for controls

Control	Stage for control	*Ct* value in the channel for fluorophore	
		FAM	ROX
C–	DNA extraction	< boundary value	Absent
NCA	PCR	Absent	Absent
C+	PCR	< boundary value	< boundary value

Table 7

Boundary *Ct* values

Sample	Plate-type instruments		Rotor-type instruments	
	Channel for fluorophore			
	FAM	ROX	FAM	ROX
C+	< 37	< 38	< 34	<35
C-	< 34	absent	< 31	absent
NCA	absent		absent	
Test samples	<36	<43	< 32	<42

10 TROUBLESHOOTING

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

1. The *Ct* value determined for the Positive Control of Amplification (C+) in the channels for the FAM and/or ROX fluorophores is greater than the boundary *Ct* value or absent, the amplification and detection should be repeated for all samples in which the specific DNA was not detected.
2. The *Ct* is determined for the Negative Control of Extraction (C–) in the channel for the ROX fluorophore, the contamination of laboratory with amplification fragments or contamination of reagents, test samples is probable at any stage of PCR analysis. Measures for detecting and elimination of contamination source must be taken. The PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples in which specific DNA was detected.
3. The *Ct* value is determined for the Negative Control of Amplification (NCA) in the channels for the FAM and/or ROX fluorophores, the contamination of laboratory with amplification fragments or contamination of reagents, test samples is probable at any stage of PCR analysis. Measures for detecting and elimination of contamination source must be taken. The amplification and detection should be repeated for all samples in which specific DNA was detected.
4. The *Ct* value is determined for the test sample, whereas the area of typical exponential growth of fluorescence is absent (the graphic looks like approximate straight line). It is necessary to check that threshold line or parameters of threshold line measurement are correct. If the result has been obtained with the correct threshold line level, the amplification and detection should be repeated for this sample.

11 TRANSPORTATION

eSens Borrelia miyamotoi QL PCR kit should be transported at 2–8 °C for no longer than 5 days. PCR kit can be transported at 2–25 °C for no longer than 3 days.

12 STABILITY AND STORAGE

All components of the **eSens Borrelia miyamotoi QL PCR kit** are to be stored at 2–8 °C when not in use (except for PCR-mix-FL *Borrelia miyamotoi* and PCR-buffer-H). All components of the **eSens Borrelia miyamotoi 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: PCR-mix-FL *Borrelia miyamotoi* and PCR-buffer-H are to be stored at the temperature from minus 24 to minus 16 °C

NOTE: PCR-mix-FL *Borrelia miyamotoi* is to be kept away from light

13 SPECIFICATIONS

13.1 Analytical sensitivity (limit of detection)

Table 8

Biological material	The volume of sample for extraction, µl	Nucleic acid extraction kit	PCR kit	Analytical sensitivity (limit of detection), copies/ml
Blood	Pellet + 100	RIBO-prep	ES3701B	10^3
Tissue (autopsy, biopsy) material	100	RIBO-prep	ES3701B	10^3
Cerebrospinal fluid	Pellet + 100	RIBO-prep	ES3701B	10^3
Ticks	100	RIBO-prep	ES3701B	10^3
		ePure Bacterial DNA Extraction Kit	ES3701B	10^3

13.2. Analytical specificity

The analytical specificity of **eSens Borrelia miyamotoi 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 reagent kit detects of DNA fragments claimed microorganisms. The analytical specificity of the reagent kit was proved in the study of the following strains of microorganisms: *Borrelia afzelii*, *B. garinii*, *B. burgdorferi sensu stricto, Leptospira interrogans*, *Coxiella burnetii*, *Bartonella quintana*, *B. henselae*, *Rickettsia conorii*, *R. sibirica*, *Babesia microti*, *Treponema pallidum*, and human genomic DNA.

When testing the DNA samples of the above microorganisms, and human DNA, DNA of ticks and DNA of rodents of nonspecific reactions was not revealed.

The clinical specificity of **eSens Borrelia miyamotoi QL PCR kit** was confirmed in laboratory clinical trials.

13.3. Diagnostic characteristics

To evaluate of diagnostic characteristics were used:

- 131 blood samples, obtained from the patients with suspected disease of ixodes tick-borne borreliosis,
- 200 samples of *I.ricinus* mites;
- 50 samples each of cerebrospinal fluid and autopsy material from the patients with diseases of a different etiology;
- 20 model samples each of cerebrospinal fluid, autopsy material and ticks, contaminated with the strain *Borrelia miyamotoi* Izh-4 from the State Collection of Pathogenic Microorganisms and Cell Cultures.

A reagent kit for *Borrelia miyamotoi* DNA detection with real-time polymerase chain reaction ((H2842-50FRT Borrelia miyamotoi Real-TM) produced by Sacace Biotechnologies Srl, Italy, was used as the kit for comparison.Table 9

Diagnostic characteristics of eSens Borrelia miyamotoi QL PCR kit

Samples type	Diagnostic sensitivity*, (with a confidence level of 90 %) in the interval (%)	Diagnostic specificity**, (with a confidence level of 90 %) in the interval (%)
Blood	91.2 – 100.0	94.0 – 99.8
Ticks	87.1 – 100.0	98.1 – 100.0
Tissue (autopsy, biopsy) material	83.9 – 100.0	92.9 – 100.0
Cerebrospinal fluid	83.9 – 100.0	92.9 – 100.0

***** Diagnostic sensitivity in comparison with a PCR reagent kit.

****** Diagnostic specificity in comparison with a PCR reagent kit.

14 QUALITY CONTROL

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

15 KEY TO SYMBOLS USED

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

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01_04/2022		

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