ECOLI

eSens

CE

IVD

For Professional Use Only

eSens Brucella sp. QL PCR kit

REF ES3807B

Instructions for Use

1 INTENDED USE

eSens Brucella sp. QL PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of DNA of *Brucella* species (*B.melitensis*, *B.abortus*, *B.suis*, *B.ovis*, *B.canis*, and *B.neotomae*) in human (whole blood, synovial fluid, and lymph node aspirate) and animal (blood, milk, placenta, lymph nodes, spleen, aborted fetal liver, hygroma, and parenchymal organs) biological materials and bacterial culture 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

Brucella spp. DNA detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *Brucella* spp. 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 the fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run.

eSens Brucella sp. QL PCR kit is a qualitative test that contains the Internal Control (Internal Control STI-704 (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 Brucella sp. QL PCR kit 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 using a wax layer. Wax melts and reaction components mix only at 95 °C.

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

Table 1

Channel for fluorophore	FAM	JOE
DNA-target	Internal Control STI-704 DNA	*Brucella* DNA
Target gene	Artificially synthesized sequence	Gene fragment (*wbopA*)

3 CONTENT

eSens Brucella sp. QL PCR kit (ES3807B) includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FEP/FRT *Brucella* spp. ready-to-use single-dose test tubes (under wax)	colorless clear liquid	0.008	55 tubes of 0.2 ml
PCR-mix-2-FL	colorless clear liquid	0.77	1 tube
Positive Control DNA *Brucella* ($C+_{Brucella}$)	colorless clear liquid	0.1	1 tube
Positive Control STI (CS+)	colorless clear liquid	0.1	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C-)*	colorless (or straw colored) clear liquid	1.6	1 tube
Internal Control STI-704 (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** during the DNA extraction procedure directly to the sample/lysis mixture.

eSens Brucella sp. QL PCR kit is intended for 55 reactions (including controls).

4 ADDITIONAL REQUIREMENTS

- DNA 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 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 tubes for PCR (0.1- or 0.2-ml; 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 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.
- Safety Data Sheets (SDS) are available on request.
- 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 Brucella sp. QL PCR kit is intended for analysis of DNA extracted with DNA extraction kits from human (whole blood, synovial fluid, and lymph node aspirate) and animal (blood, milk, placenta, lymph nodes, spleen, aborted fetal liver, hygroma, and parenchymal organs) biological materials and bacterial culture:

Sampling

Human material:

6.1 Whole peripheral blood.

Whole peripheral blood is collected into the tubes with 3 % EDTA solution (50 µl of EDTA solution per 1 ml of blood).

6.2 Lymph node aspirate.

Lymph node aspirate is collected into sterile tubes with 100 µl of sterile 0.9 % NaCl solution or transport medium (manufactured by FBIS CRIE).

6.3 Synovial fluid.

Synovial fluid is collected to a sterile disposable tube.

Animal material:

6.4 Whole blood.

Whole blood (5 ml). Blood is collected after overnight fasting into Vacuette® tubes with 6 % EDTA solution (50 µl of EDTA solution per 1 ml of blood). Close the tubes with blood and overturn them gently several times (to mix the content).

6.5 *Exudate from lesion foci*.

Exudate from lesion foci (in case of skin form) should be placed to 200 µl of 0.9 % sterile saline solution and is used without pretreatment.

6.6 Sputum.

Sputum should be treated with **Mucolysin** reagent (180-CE) according to the Mucolysin *Instruction manual*. If it is necessary to repeat the analysis, the rest of pretreated sputum should be frozen.

6.7 Blood.

Blood is collected to tubes with 6 % EDTA solution (50 µl of EDTA solution per 1 ml of blood).

6.8 *Milk*.

Milk (10-20 ml) is collected to sterile vessel.

6.9 *Abdominal and stomach fluids, spleen and liver of aborted fetus*.

6.10 Placenta and fetal membranes of aborted animals.

6.11 Fluid of bursa, hydroma.

6.12 in case of *animal slaughter*, *whole pair lymph nodes* (*para-aortic*, *supramammary*, *inguinal* and *pelvic*) from both sides of the carcass, parts of *parenchymatous organs* (*liver*, *spleen*), *testicles* with epididymes obtained from males with signs of orchitis or epididymitis are collected for analysis.

Bacterial cultures:

6.13 Liquid cultures.

Liquid cultures are used without pretreatment.

6.14 *Bacterial colonies*.

Bacterial colonies suspicious for *Brucell*a spp should be resuspended in 0.5 ml of sterile saline.

The material can be stored at 2–8 °C for 1 day and at the temperature below minus 16 °C for 1 month. Only one freeze-thawing cycle is acceptable

Pretreatment

6.15 Whole blood, synovial fluid, lymphatic node aspirate, fluid of bursa and hydroma, and microorganism cultures.

Samples of whole blood preserved in EDTA, synovial fluid, lymphatic node aspirate, fluid of bursa and hydroma, and microorganism cultures are used for DNA extraction without pretreatment after disinfection procedure.

6.16 Parenchymal organs, testicles, placenta, fetal membranes and lymph nodes.

Homogenize the samples of parenchymal organs, testicles, placenta, and fetal membranes (separately) by size 1x1x1 cm and whole lymph nodes by trituration using sterile porcelain mortar and mallet. Then add equal volume of sterile saline and mix carefully. Incubate at 20–25 °C for 5 min. Transfer 0.4-0.5 ml of the upper phase to a 1.5-ml tube with a pipette using a tip with aerosol barrier, disinfect it and use 0.1 ml for DNA extraction. Utilize the tube with a hypophase.

6.17 Milk.

Centrifuge 10 ml of milk after disinfection procedure at 3000 rpm for 10–15 min. If the pellet is practically invisible, add another 10 ml of milk to the same tube and repeat the centrifugation. Discard the supernatant leaving about 200 µl of liquid above the pellet. Resuspend the pellet in this liquid and use 0.1 ml of the suspension for DNA extraction.

Disinfection:

1. Add 0.1 % sodium merthiolate (1 : 1000 dilution) to a final concentration of 0.01 % (1 : 10000 dilution) to biological material samples and bacterial cultures (if it is required after preliminary treatment) and warm up at the temperature (56 ± 1) °C for 30 min. Use 100 µl of the prepared samples for further tests.
2. Transfer 1 ml of suspect bacterial colonies treated with sodium merthiolate to 1.5-ml tubes and centrifuge at 12000 rpm for 15 min. Discard the supernatant, resuspend the pellet in 100 µl of 0.9 % NaCl and use it in further work.
3. **Lysis Solution** from **DNA-sorb-B** kit (K1-2-100-CE) (if it has been stored at 2–8 °C) should be heated at 65 °C until complete crystal dissolution.
4. Add 300 µl of Lysis Solution to each tube with disinfected material (100 µl) and incubate at 65 °C for 15 min.

Further analysis is performed according to the **DNA-sorb-B** protocol.

7 WORKING CONDITIONS

eSens Brucella sp. QL PCR kit should be used at 18–25 °C.

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

- **DNA-sorb-B** (K1-2-100-CE)

- 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 STI-704 (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 DNA sample is **10 µl**.

1. Prepare the required number of tubes with **PCR-mix-1-FEP/FRT *Brucella* spp.** and wax for amplification of DNA from clinical and control samples.
2. Add **7 µl** of **PCR-mix-2-FL** to the surface of the wax layer into each tube ensuring that it does not fall under the wax and mix with **PCR-mix-1-FEP/FRT *Brucella* spp.**
3. Add **10 µl** of **DNA samples** obtained at the DNA extraction stage.
4. Carry out the control reactions:

NCA	–	Add **10 µl** of **DNA-buffer** to the tube labeled **NCA** (Negative Control of Amplification).
C+$_{Brucella}$	–	Add **10 µl** of **Positive Control DNA Brucella (C+$_{Brucella}$)** to the tube labeled **C+$_{Brucella}$** (Positive Control of Amplification).
CS+	–	Add **10 µl** of **Positive Control STI (CS+)** to the tube labeled **CS+** (Positive Control of Amplification).
C–	–	Add **10 µl** of **the sample extracted from the Negative Control (C–) reagent** to the tube labeled C– (Negative control of Extraction).

8.2.2 Amplification

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

Table 2

eSens 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**	**Cycles**
1	**95**	**5 min**	**1**
2	**95**	**10 s**	**10**
	65	**25 s**	
	72	**10 s**	
3	**95**	**10 s**	**35**
	56	**25 s Fluorescence acquiring**	
	72	**10 s**	

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

1. Adjust the fluorescence channel sensitivity.
2. Insert tubes into the reaction module of the device.
3. Run the amplification program with fluorescence detection.
4. Analyze results after the amplification program is completed.

8.3 Instrument Settings

Test settings for rotor-type and plate-type instruments

Channel	**Calibrate/Gain Optimisation**	**Threshold**	**Dynamic tube**	**Slope Correct**	**More Settings/ Outlier Removal**
FAM/Green	from 5 FI to 10 FI	0.1	On	-	0 %
JOE/Yellow	from 5 FI to 10 FI	0.1	On	-	10 %

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 IC DNA amplification product is detected in the channel for the FAM fluorophore.
- The signal of the *Brucella* DNA 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:

1. *Brucella* spp. DNA is **detected** if the *Ct* value determined in the results grid in the channel for the JOE fluorophore is less than the specified boundary Ct value.
2. *Brucella* spp. DNA is **not detected** in a sample if the *Ct* value is not determined (absent) in the channel for the JOE fluorophore, whereas the *Ct* value determined in the channel for the FAM fluorophore is less than the specified boundary Ct value.

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 and Table 4).

Table 3

Results for controls

Control	Stage for control	*Ct* value in the channel for fluorophore	
		FAM	JOE
C–	DNA extraction	<boundary value	Absent
NCA	PCR	Absent	Absent
C+$_{Brucella}$	PCR	Absent	<boundary value
CS+	PCR	<boundary value	Absent

Table 4

Boundary *Ct* values

Sample	Channel	*Ct* value
C–	FAM/Green	31
C+$_{Brucella}$	JOE/Yellow	33
CS+	FAM/Green	31
Test samples	FAM/Green	31
	JOE/Yellow	33

10 TROUBLESHOOTING

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

1. If the *Ct* value is absent for the Positive Controls of Amplification (C+), this indicates incorrectly chosen amplification program or other errors of amplification stage. PCR should be repeated.
2. If the *Ct* value determined in the channel for the JOE fluorophore is greater than the boundary value, whereas the *Ct* value determined for the FAM fluorophore is less than the specified boundary *Ct* value, PCR should be repeated. The result of analysis is **positive** if the same result has been obtained or if the *Ct* value determined in the channel for the JOE fluorophore is less than the specified boundary *Ct* value.
3. If the *Ct* value determined in the channel for the JOE fluorophore is absent, whereas the *Ct* value determined in the channel for the FAM fluorophore is greater than the boundary value or

absent, PCR and detection should be repeated. If the same result has been obtained, it is necessary to repeat the analysis of the sample beginning with the DNA extraction stage.

4. If any *Ct* value is determined for the Negative Control of Extraction (C–) in the channel for the JOE fluorophore and for the Negative Control of amplification (NCA) (DNA-buffer) in any channel, it indicates contamination of reagents or samples. In this case, the results of analysis for all samples are **invalid**. The analysis for all samples should be repeated and measures for detecting and elimination of contamination source must be taken.

11 TRANSPORTATION

eSens Brucella sp. QL PCR kit should be transported at 2–8 °C for no longer than 5 days.

12 STABILITY AND STORAGE

All components of the **eSens Brucella sp. QL PCR kit** are to be stored at 2–8 °C when not in use. All components of the **eSens Brucella sp. QL PCR kit** are stable until the expiry date on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: PCR-mix-1-FEP/FRT *Brucella* spp. is to be stored away from light.

13 SPECIFICATIONS

13.1 Sensitivity

Analytical sensitivity of **eSens Brucella sp. QL PCR kit** is no less than $1x10^3$ bacterial cells per 1 ml of sample.

NOTE: The claimed analytical features of **eSens Brucella sp. QL PCR kit** are guaranteed only when additional reagents kit **DNA-sorb-B** (manufactured by FBIS CRIE) is used.

13.2 Specificity

The analytical specificity of **eSens Brucella sp. QL PCR kit** is ensured by selection of specific primers and probes, as well as stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

The clinical specificity of **eSens Brucella sp. QL PCR kit** was confirmed in laboratory clinical trials.

14 QUALITY CONTROL

In compliance with Federal Budget Institute of Science "Central Research Institute for Epidemiology" ISO 13485-Certified Quality Management System, each lot of **eSens Brucella sp. QL PCR kit** has been tested against predetermined specifications to ensure consistent product quality.

15 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+**	Positive control of amplification
CS+	Positive control STI	**IC**	Internal control
FBIS CRIE	Federal Budget Institute of Science "Central Research Institute for Epidemiology"	**C+*Brucella***	Positive Control of Amplification

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