



For in Vitro Diagnostic Use

AmpliSens® *Brucella* spp.-FEP PCR kit

Instruction Manual



TABLE OF CONTENTS

1. INTENDED USE.....	2
2. PRINCIPLE OF PCR DETECTION.....	2
3. CONTENT.....	2
4. ADDITIONAL REQUIREMENTS.....	2
5. GENERAL PRECAUTIONS.....	3
6. SAMPLING AND HANDLING.....	3
7. PROTOCOL.....	4
8. DATA ANALYSIS.....	5
9. TROUBLESHOOTING.....	5
10. STABILITY AND STORAGE.....	6
11. SPECIFICATIONS.....	6
12. REFERENCES.....	6
13. QUALITY CONTROL.....	6
14. EXPLANATION OF SYMBOLS.....	6



Ecoli s.r.o., Studenohorská 12
841 03 Bratislava 47
Slovak Republic
Tel.: +421 2 6478 9336
Fax: +421 2 6478 9040
ecoli@ecoli.sk
www.ecoli.sk www.pcrdiagnostics.eu



Federal State Institution of Science
Central Research Institute of Epidemiology
3A Novogireevskaya Street
Moscow 111123 Russia

1. INTENDED USE.

AmpliSens® *Brucella* spp.-FEP PCR kit is an in vitro nucleic acid amplification test for qualitative detection of *Brucella* species (*B.melitensis*, *B.abortus*, *B.suis*, *B.ovis*, *B.canis*, *B.neotomae*) DNA in the human (whole blood, synovial fluid, lymph node punctate) and animal (blood, milk, placenta, lymph nodes, spleen, liver of aborted fetus, hygroma, parenchymal organs) biological materials, and bacterial culture by using end-point hybridization-fluorescence detection of amplified products.

2. PRINCIPLE OF PCR DETECTION.

Brucella spp. detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special *Brucella* spp. primers. In Fluorescent End-Point PCR, the amplified product is detected using fluorescent dyes. These dyes are usually linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. A multi channel rotor-type fluorometer is specially designed to detect fluorescent excitation from the fluorophores in a reaction mix after PCR. It allows the accumulating product detection without re-opening the reaction tubes after the PCR run. AmpliSens® *Brucella* spp.-FEP PCR kit is a qualitative test, which contains the Internal Control (IC). It 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® *Brucella* spp.-FEP 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 wax layer. Wax melting and reaction mix components occur only at 95 °C.

3. CONTENT.

AmpliSens® *Brucella* spp.-FEP PCR kit is produced in 2 forms:

AmpliSens® *Brucella* spp.-FEP PCR kit (vials 0.5 ml), **REF** B10-R0,5-FEP-CE.

AmpliSens® *Brucella* spp.-FEP PCR kit (vials 0.2 ml), **REF** B10-R0,2-FEP-CE.

AmpliSens® *Brucella* spp.-FEP PCR kit includes:

Reagent	Description	Volume (ml)	Amount
PCR-mix-1-FEP/FRT <i>Brucella</i> spp. ready-to-use single-dose test tubes (under wax)	colorless, clear liquid	0.008	55 tubes of 0.5 or 0.2 ml
PCR-mix-2-FL	colorless, clear liquid	0.77	1 tube
PCR-mix-Background	colorless, clear liquid	0.5	1 tube
Mineral oil for PCR	colorless viscous liquid	2.0	1 dropper bottle
Positive Control DNA <i>Brucella</i> (C+)	colorless, clear liquid	0.1	1 tube
DNA-buffer	colorless, clear liquid	0.5	1 tube
Negative Control (C-)*	colorless, clear liquid	1.2	1 tube
Internal Control STI-704**	colorless, clear liquid	0.5	1 tube

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

** add 10 µl of Internal Control during the DNA isolation procedure directly to the sample/lysis mixture ("DNA-sorb-B", **REF** K1-2-50-CE protocol).

AmpliSens® *Brucella* spp.-FEP PCR kit is intended for 55 reactions, including controls.

4. ADDITIONAL REQUIREMENTS.

- DNA isolation kit.
- Disposable powder-free gloves and laboratory coat.
- 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.
- Personal thermocyclers (for example, Gradient Palm Cycler (Corbett Research, Australia), GeneAmp PCR System 2700 or GeneAmp PCR System 2400 (Applied Biosystems, USA), Uno-2 (Biometra, Germany), MiniCycler, PTC-100 (MJ Research, USA) or equivalent).

- Fluorometer (for example, ALA-1/4 (Biosan, Latvia) or equivalent).
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity .
- Refrigerator for temperature between 2 and 8 °C.
- Deep-freezer with temperature not more than 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 barriers and use new tip for every procedure.
- Store and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, protect eyes while samples and reagents handling. Thoroughly wash hands afterward.
- 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 samples and unused reagents in compliance with local authorities requirements.
- Samples 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 contact with the skin, eyes and mucosa. If skin, eyes and mucosa contact immediately flush with water, seek medical attention.
- 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.
- The laboratory process must be one directional, it should begin in the Extraction Area move 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 samples of biological materials for PCR-analysis, transportation and storage is described in manufacturer's handbook [2]. It is recommended that this handbook is read before starting work.

AmpliSens® *Brucella* spp.-FEP PCR kit is intended for analysis of DNA extracted by using DNA isolation kits from:

Samples from human:

- *whole peripheral blood* is collected in tubes with 3 % EDTA solution (50 µl of EDTA per 1 ml of blood).
- *lymphatic nodes puncture sample* is collected in tubes with 100 µl of sterile 0.9 % sodium chloride solution or transport medium (manufactured by Federal State Institution of Science Central Research Institute of Epidemiology).
- *synovial fluid* is collected in a sterile disposable tube.

Samples from animals:

- *blood* is collected in tubes with 6 % EDTA solution (50 µl of EDTA per 1 ml of blood) and used for DNA isolation after decontamination procedure.
- *Milk* (10-20 ml) is collected in sterile vessel.
- *abdominal and stomach fluids, spleen and liver of aborted fetus.*
- *placenta and fetal membranes of aborted animals.*
- *fluid of bursa, hydroma.*
- in case of animals slaughter whole pair lymphatic nodes (paraortic, inguinal, pelvic) from both sides of carcass, parts of parenchymatous organs (spleen,liver), seminal gland with appendages from male with stigmas of orchitis or epididymitis are collected for analysis.

Bacterial cultures:

- *liquid cultures* are used without preliminary treatment.
- *suspicious bacterial colonies* should be resuspended in 0.5 ml of sterile physiological solution).

Material for analysis can be stored at 2–8 °C for 1 day and at minus 16 °C for 1 month.



Only one freeze-thaw cycle of clinical material is allowed.

Preliminary treatment of material.

Samples of whole blood, preserved in EDTA, synovial fluid, lymphatic nodes punctuate, fluid of bursa and hydroma, microorganism cultures are used for DNA isolation without preliminary treatment after decontamination procedure.

Samples of parenchymatous organs, seminal gland, placenta and fetal membranes (separately) by size 1x1x1 cm and whole lymphatic nodes are homogenized in sterile porcelain mortars with pestle. Then add equal volume of sterile physiological solution and mix. Incubate

5 min at the temperature from 20 to 25 °C. Transfer 0.4-0.5 ml of the upper phase to the 1.5 ml tube by pipette tip with aerosol barrier, decontaminate it and use 0.1 ml for DNA isolation. Utilize the bottom phase with tube.

Centrifuge 10 ml of milk after decontamination procedure at 3000 rpm for 10-15 min. If sediment is practically not visible, add above 10 ml of material in the same tube and repeat the centrifugation. Discard the supernatant and leave on sediment about 200 µl of liquid.

Resuspend sediment in remained volume of supernatant. Use 0.1 ml of suspension for DNA extraction.

Decontamination procedure.

1. In sample of the biological material and bacterial cultures (if it is required after preliminary treatment) add 0.1 % sodium mertiolate (1:1000 dilution) up to final concentration of 0.01 % (1:10000 dilution) and warm up at the temperature (56±1) °C for 30 min. Use 0.1 ml of prepared samples for further tests.

2. Transfer 1 ml of suspicious bacterial colonies, treated by sodium mertiolate, into the 1.5 ml tubes and centrifugate 15 min at 12000 rpm. Discard supernatant, resuspend sediment in 100 µl of 0.9 % solution of sodium chloride and use it in further work.

3. Lysis Solution from "DNA-sorb-B", [REF](#) K1-2-50-CE (if stored at 2-8 °C) should be heated to 65 °C until ice crystals disappear.

4. Add 300 µl of Lysis Solution into each tube with 100 µl of decontaminated material and incubate at 65 °C for 15 min.

Further analysis is performed according "DNA-sorb-B", [REF](#) K1-2-50-CE Protocol.

7. PROTOCOL.

7.1. DNA Isolation

It's recommended to use the following nucleic acid extraction kits:

- "DNA-sorb-B", [REF](#) K1-2-50-CE.



Carry out the DNA isolation according to the manufacturer's instructions.



Centrifugation speed after adding of **Universal Sorbent** and **Washing Solution 1** is 8000-10000 rpm (10000-13000 rpm in case of rotor radius r = 70 mm).

7.2. Preparing the PCR.

Total reaction volume - **25 µl**, volume of DNA sample - **10 µl**.

7.2.1 Preparing tubes for PCR.

1. Prepare the required number of tubes with **PCR-mix-1-FEP/FRT *Brucella* spp.** with 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 of each tube ensuring that it does not fall under the wax and mix with **PCR-mix-1-FEP/FRT *Brucella* spp.**
3. Add above **1 drop** of **mineral oil for PCR** (about **25 µl**).
4. Prepare 2 tubes with **PCR-mix-1-FEP/FRT *Brucella* spp.** and mark them as **Background**. Add **17 µl** of **PCR-mix-Background** to the surface of the wax layer of each tube, ensuring that it does not fall under the wax and mix with **PCR-mix-1-FEP/FRT *Brucella* spp.** Add above **1 drop** of **mineral oil for PCR**.
5. Using tips with aerosol barrier add **10 µl** of **DNA samples** obtained from clinical or control samples at the DNA extraction stage.
6. Carry out the 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 DNA <i>Brucella</i> to the tube labeled C+ (Positive Control of Amplification).

7.2.2 Amplification.

Run the following program on the thermocycler (see Table 1). When the temperature reaches 95 °C (pause regimen), insert tubes to cells of amplifier and press the button to continue.

It is recommended to sediment drops from walls of tubes by short vortex (1–3 sec) before placing them in the thermocycler.

Table 1

Programming thermocyclers for DNA amplification of *Brucella* spp.

цикл	Thermocyclers with active temperature adjustment						Thermocyclers with block temperature adjustment: "Uno-2" (Biomtra), "MiniCycler", "PTC-10"» (MJ Research)		
	"GeneAmp PCR System 2400" (ABI)			"GeneAmp PCR System 2700" (ABI), "Gradient Palm Cycler" (Corbett Research)			Tempe-rature	Time	Cycles
0	95 °C	pause		93 °C	pause		95 °C	pause	
1	95 °C	2 min	1	93 °C	2 min	1	95 °C	2 min	1
2	95 °C	10 sec	10	93 °C	10 sec	10	95 °C	25 sec	10
	65 °C	25 sec		65 °C	25 sec		65 °C	40 sec	
	72 °C	10 sec		72 °C	25 sec		72 °C	25 sec	
3	95 °C	10 sec	35	93 °C	10 sec	35	95 °C	25 sec	35
	56 °C	25 sec		56 °C	25 sec		56 °C	40 sec	
	72 °C	10 sec		72 °C	25 sec		72 °C	25 sec	
4	10 °C	storage		10 °C	storage		10 °C	storage	

8. DATA ANALYSIS.

Detection is conducted on ALA-1/4 fluorescence detector.



Please read Aladin Operating Manual before use of this kit.

Program the detector according to the manufacturer's manual and Appendix 1.

8.2. Results interpretation.

1. When the analysis is complete the results are automatically shown in the table as follows:

pos – positive result;

neg – negative result;

eq – equivocal result (signal is in grey zone);

nd – invalid result (specific signal and IC signal are absent in the sample).

2. Result of the analysis is considered reliable only if both Positive and Negative Controls of amplification as well as Negative Control of extraction are passed (Table 2).

Table 2

Results for controls

Control	Stage for control	Result of automatic interpretation		Interpretation
		FAM channel (IC)	HEX channel (samples)	
C-	DNA isolation	+	<i>Brucella</i> - neg	OK
NCA	Amplification	-	<i>Brucella</i> - nd	OK
C+	Amplification	-	<i>Brucella</i> – pos	OK

9. TROUBLESHOOTING.

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

- Samples with **nd** result (except for NCA) are irrelevant and require repeating of PCR and detection. If **nd** result is obtained in the second run, the sample should be examined starting from the stage of DNA extraction. For NCA **nd** result is valid.
- Samples with **eq** (equivocal) result are irrelevant and require repeating of PCR and detection. If the same result is obtained in the second run, these samples should be considered as positive.
- No positive signal in positive control of PCR (C+) can indicate incorrect programming of the temperature profile of the thermocycler, incorrect configuration of the PCR reaction, or storage conditions for kit components did not comply with manufacturer instruction, or reagents kit has expired. It is necessary to check programming of the thermocycler (see 7.2.2.), storage conditions, and the expiration date of the reagents and repeat PCR reaction once again for all samples.
- Positive signal in negative controls (C- or NCA) indicates contamination of reagents or samples. In such case results of analysis must be considered as irrelevant. Test analysis must be repeated and measures for detecting of contamination source must be undertaken.

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

10. STABILITY AND STORAGE.

All components of the **AmpliSens® *Brucella* spp.-FEP** PCR kit are to be stored at the temperature between 2 and 8 °C, when not in use. All components of the **AmpliSens® *Brucella* spp.-FEP** PCR kit are to be stable until labeled expiration date.

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens® *Brucella* spp.-FEP** PCR kit is no less than 1x10³ bacterial cells per 1 ml of sample.



The claimed analytical features of **AmpliSens® *Brucella* spp.-FEP** PCR kit are guaranteed only when additional reagents kit "DNA-sorb-B" (manufactured by Federal State Institution of Science Central Research Institute of Epidemiology) is used.

11.2. Specificity.

Specificity of **AmpliSens® *Brucella* spp.-FEP** PCR kit is ensured by selection of specific primers and probes, as well as the selection of strict reaction conditions. The primers and probes have been checked for possible homologies to all in gene banks published sequences by sequence comparison analysis. Specificity of **AmpliSens® *Brucella* spp.-FEP** PCR kit was confirmed in laboratory clinical trials.

12. REFERENCES.

1. Debeaumont C., Falconnet P.A., Maurin M. Real-time PCR for detection of *Brucella* spp., DNA in human serum samples. Eur.J.Clin.Microbiol.Infect Dis. 2005 Dec, 24 (12):842-845.
2. 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® *Brucella* spp.-FEP** 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