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For Professional Use Only

AmpliSens® *Brucella* spp.-FEP

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® *Brucella* spp.-FEP 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, B.neotomae)* in human (whole blood, synovial fluid, lymph node punctate) and animal (blood, milk, placenta, lymph nodes, spleen, aborted fetal liver, hygroma, and parenchymal organs) biological materials and bacterial culture by using end-point hybridization-fluorescence detection of amplified products.

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 pathogen genome specific region using specific *Brucella* spp. primers. In **F**luorescent **E**nd-**P**oint PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes that bind specifically to the amplified product during thermocycling. A multichannel rotor-type fluorometer is specially designed to detect fluorescent emission from the fluorophores in the reaction mixture after PCR. It allows detection of the accumulating product without re-opening the reaction tubes after the PCR run. **AmpliSens® *Brucella* spp.-FEP** PCR kit is a qualitative test that 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 the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using a wax layer. Wax melts and reaction mixture components mix only at 95 °C.

3. CONTENT

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

AmpliSens® *Brucella* spp.-FEP PCR kit (0.5-ml vials), **REF** B10-50-R0,5-FEP-CE.

AmpliSens® *Brucella* spp.-FEP PCR kit (0.2-ml vials), **REF** B10-50-R0,2-FEP-CE.

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

Reagent	*Description*	*Volume (ml)*	*Amount*
PCR-mix-1-FEP/FRT *Brucella* spp. ready-to-use single-dose test tubes (*under wax)*	colorless clear liquid	0.008	55 tubes of 0.5 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 *Brucella* (C+$_{Brucella}$)	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	1.2	1 tube
Internal Control STI-704 (IC)**	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 STI-704 (IC) 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 a 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 (0.5- or 0.2 ml; for example, Axygen, USA).
- Refrigerator for 2–8 °C
- Deep-freezer for ≤ –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 another suitable disinfectant.
- Avoid contact with the skin, eyes, and mucosa. If skin, eyes, or mucosa contact, immediately flush with water and 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 are 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 to tubes with 3 % EDTA (50 µl of EDTA per 1 ml of blood).

- *Lymph node aspirate* is collected to tubes with 100 µl of sterile 0.9 % NaCl or with transport medium (manufactured by Federal Budget Institute of Science “Central Research Institute for Epidemiology”).
- *synovial fluid* is collected to a sterile disposable tube.

Samples from animals:

- *blood* is collected to tubes with 6 % EDTA (50 µl of EDTA per 1 ml of blood) and used for DNA extraction after decontamination procedure.
- *Milk* (10-20 ml) is collected to a sterile vessel.
- *abdominal and stomach fluids, aborted fetal spleen and liver.*
- *placenta and fetal membranes of aborted animals*.
- *fluid of bursa, hydroma*.
- in case of animals, slaughter whole pair lymph nodes (paraaortic, inguinal, and pelvic) from both sides of the carcass, parts of parenchymal organs (spleen and liver), testicles with epididymes obtained from males with signs of orchitis or epididymitis are collected for analysis.

Bacterial cultures:

- *liquid cultures* are used without pretreatment.
- *suspect bacterial colonies* should be resuspended in 0.5 ml of sterile saline).

Material for analysis can be stored at 2–8 °C for 1 day and at –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, lymph node aspirate, fluid of bursa and hydroma, and microorganism cultures are used for DNA isolation without pretreatment after decontamination procedure.

Pieces (~ 1 x 1 x 1 cm) of parenchymal organs, testicles, placenta, and fetal membranes (separately) and whole lymph nodes are homogenized in sterile porcelain mortars with pestle. Then add an equal volume of sterile saline and mix. 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, decontaminate it, and use 0.1 ml for DNA isolation. Utilize the bottom phase with a tube.

Centrifuge 10 ml of milk after decontamination 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 centrifugation. Discard the supernatant leaving ~200 µl of liquid above the pellet. Resuspend the pellet in this liquid and use 0.1 ml of the suspension for DNA extraction.

Decontamination procedure

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 0.1 ml 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, 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 to each tube with 100 µl of decontaminated material and incubate at 65 °C for 15 min.

Further analysis is performed according to the DNA-sorb-B, REF K1-2-50-CE Protocol.

7. WORKING CONDITIONS

AmpliSens® *Brucella* spp.-FEP PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA Isolation

It is recommended to use the following nucleic acid extraction kits:

- DNA-sorb-B, REF K1-2-50-CE.

Extract DNA according to the manufacturer's instructions.

After adding **Universal Sorbent** and **Washing Solution 1**, centrifuge samples at 8000–10000 rpm (10000–13000 rpm if a rotor with a radius of 70 mm is used).

8.2. Preparing PCR

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

8.2.1 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** (~ **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+$_{Brucella}$ - Add **10 µl** of **Positive Control DNA *Brucella*** to the tube labeled C+$_{Brucella}$ (Positive Control of Amplification).

8.2.2 Amplification

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

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

Table 1

Programming thermocyclers for *Brucella* spp. DNA amplification

	Thermocyclers with active temperature adjustment						Thermocyclers with block temperature adjustment: **Uno-2 (Biometra), MiniCycler, PTC-10 (MJ Research)**		
	GeneAmp PCR System 2400 (Applied Biosystems)			**GeneAmp PCR System 2700 (Applied Biosystems), Gradient Palm Cycler (Corbett Research)**					
цикл	Tempe-rature	Time	Cycles	Tempe-rature	Time	Cycles	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 s	10	**93 °C**	10 s	10	**95 °C**	25 s	10
	65 °C	25 s		**65 °C**	25 s		**65 °C**	40 s	
	72 °C	10 s		**72 °C**	25 s		**72 °C**	25 s	
3	**95 °C**	10 s	35	**93 °C**	10 s	35	**95 °C**	25 s	35
	56 °C	25 s		**56 °C**	25 s		**56 °C**	40 s	
	72 °C	10 s		**72 °C**	25 s		**72 °C**	25 s	
4	**10 °C**	storage		**10 °C**	storage		**10 °C**	storage	

9. DATA ANALYSIS

Detection is conducted in the ALA-1/4 florescence detector.

Please read ALA-1/4 Operating Manual before using this kit.

Program the detector according to the manufacturer's manual and Guidelines.

9.2. Results interpretation

1. When the analysis is completed, 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 the results obtained for Positive and Negative Controls of amplification as well as Negative Control of extraction are correct (Table 2).

Table 2

Results for controls

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

10. TROUBLESHOOTING

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

- Samples with **nd** result (except for NCA) are irrelevant. PCR and detection should be repeated for them. If **nd** result is obtained in the second run, the sample should be examined starting from the DNA extraction stage. For NCA **nd,** the result is valid.
- Samples with **eq** (equivocal) result are irrelevant and require repeating PCR and detection. If the same result is obtained in the second run, these samples should be considered as **positive**.
- No positive signal in the positive control of PCR (C+$_{Brucella}$) may 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 8.2.2.), 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 this case, the results of analysis should 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.

11. TRANSPORTATION

AmpliSens® *Brucella* spp.-FEP PCR kit should be transported at 2–8 ºC for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *Brucella* spp.-FEP** PCR kit are to be stored at 2–8 ºC when not in use. All components of the **AmpliSens® *Brucella* spp.-FEP** PCR kit are stable

until the expiration date on the label. The shelf life of opened reagents is the same as that of unopened reagents, unless otherwise stated.

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

13. SPECIFICATIONS

13.1. Sensitivity

Analytical Sensitivity of **AmpliSens® *Brucella* spp.-FEP** PCR kit is no less than $1x10^3$ 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 Budget Institute of Science "Central Research Institute for Epidemiology") is used.

13.2. Specificity

The analytical specificity of **AmpliSens® *Brucella* spp.-FEP** PCR kit is ensured by selection of specific primers and probes and 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 **AmpliSens® *Brucella* spp.-FEP** PCR kit was confirmed in laboratory clinical trials.

14. 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 Budget Institute of Science "Central Research Institute for Epidemiology" of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being, Moscow, 2008.

15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science "Central Research Institute for 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.

16. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Sufficient for
IVD	*In vitro* diagnostic medical device		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation		Keep away from sunlight
	Manufacturer	**NCA**	Negative control of amplification
	Date of manufacture	**C–**	Negative control of extraction
EC REP	Authorised representative in the European Community	**C+*Brucella***	Positive Control of Amplification
FBIS CRIE	Federal Budget Institute of Science “Central Research Institute for Epidemiology”	**IC**	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
13.12.10	Cover page	The phrase “For Professional Use Only” was added
	Content	New sections “Working Conditions” and “Transportation” were added
		The “Explanation of Symbols” section was renamed to “Key to Symbols Used”
	Stability and Storage	The information about the shelf life of reagents before and after the first use was added
		Information that PCR-mix-1-FEP/FRT *Brucella* spp. is to be kept away from light was added
	Key to Symbols Used	The explanation of symbols was corrected
	Footer	Reference numbers were changed from B10-R0,5-FEP-CE; B10-R0,2-FEP-CE to B10-50-R0,5-FEP-CE; B10-50-R0,2-FEP-CE
	Text	Shorten name of the Positive Control DNA *Brucella* was changed from C+ to $C+_{Brucella}$
21.06.11 LA	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”