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IVD

For Professional Use Only

AmpliSens® *Brucella* spp.-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

Ecoli s.r.o., Studenohorska 12
841 03 Bratislava 47
Slovak Republic
Tel.: +421 2 6478 9336
Fax: +421 2 6478 9040

Federal Budget Institute of
Science “Central Research
Institute for Epidemiology”
3A Novogireevskaya Street
Moscow 111123 Russia

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1. INTENDED USE

AmpliSens® *Brucella* spp.-FRT 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 by using real-time hybridization-fluorescence detection.

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

2. PRINCIPLE OF PCR DETECTION

Brucella spp. DNA detection by polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using specific *Brucella* spp*.* primers. In real-time PCR, the amplified product is detected using 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. **AmpliSens® *Brucella* spp.-FRT** PCR kit is a qualitative test that contains the Internal Control (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. **AmpliSens ® *Brucella* spp.-FRT** 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 components mix only at 95 °C.

3. CONTENT

AmpliSens® *Brucella* spp.-FRT PCR kit is produced in 1 form:

AmpliSens® *Brucella* spp.-FRT PCR kit variant FRT (for use with RG), **REF** R-B10(RG)-CE.

AmpliSens® *Brucella* spp.-FRT PCR kit variant FRT 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 0.2 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-88 (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	1.2	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 (see DNA-sorb-B, **REF** K1-2-50-CE protocols).

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

4. ADDITIONAL REQUIREMENTS

- DNA extraction 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, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia).
- Disposable polypropylene microtubes for PCR (0.1- 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 and then 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.-FRT** PCR kit is intended for analysis of DNA extracted with DNA extraction 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).
- *Lymph node aspirate* is collected to tubes with 100 µl of sterile 0.9 % NaCl or 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 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 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, lymphatic node aspirate, fluid of bursa and hydroma, and microorganism cultures are used for DNA extraction without preliminary treatment after decontamination procedure.

Samples of parenchymal organs, testicles, placenta, and fetal membranes (separately) by size 1x1x1 cm and whole lymph nodes are homogenized in sterile porcelain mortars with pestle. Then add 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 extraction. 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 the 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.-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA extraction

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.** 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 of each tube ensuring that it does not fall under the wax and mix with **PCR-mix-1-FEP/FRT *Brucella* spp.**
3. Using tips with aerosol barrier add **10 µl** of **DNA** obtained from clinical or control samples at the DNA extraction stage to the prepared tubes.
4. 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).

CS+ - Add **10 µl** of **Positive Control STI-88** to the tube labeled CS+.

8.2.2. Amplification

1. Program the Rotor-Gene according to manufacturer's manual and Guidelines.
2. Create a temperature profile on your Rotor-Gene instrument as follows:

Step	*Temperature, °C*	*Time*	*Fluorescence detection*	*Cycles*
Hold	95	5 min	–	1
Cycling 1	95	10 s	–	10
	65	25 s	–	
	72	10 s	–	
Cycling 2	95	10 s	–	35
	56	25 s	FAM/Green, JOE/Yellow	
	72	10 s	–	

3. Fluorescence is detected on the 2-nd step **(56 °C)** of stage Cycling 2 in FAM/Green and

JOE/Yellow fluorescence channels.

4. Adjust the fluorescence channel sensitivity according to Guidelines.

9. DATA ANALYSIS

Internal Control is detected in the FAM/Green fluorescence channel, *Brucella* DNA is detected in the JOE/Yellow fluorescence channel.

See Guidelines for data analysis settings for Rotor-Gene 3000 or Rotor-Gene 6000.

9.1. Results interpretation

The results are interpreted by the software of Rotor-Gene 3000 or Rotor-Gene 6000 Instrument by the crossing (or not crossing) of the fluorescence curve with the threshold line.

Results for controls

Control	Stage for control	Ct channel FAM (Green)	Ct channel JOE (Yellow)	Interpretation
C–	DNA extraction	Pos (< X*)	Neg	OK
NCA	Amplification	Neg	Neg	OK
C+*Brucella*	Amplification	Neg	Pos (< Z*)	OK
CS+	Amplification	Pos (< Y*)	Neg	OK

*For X, Y, and Z values see Guidelines.

1. The sample is considered to be **positive** for *Brucella* spp. if its Ct value does not exceed Z in the JOE /Yellow channel.
2. The sample is considered to be **negative** for *Brucella* spp. if its Ct value is absent in the JOE/Yellow channel (the fluorescence curve does not cross the threshold line) and the Ct value in the FAM/Green channel does not exceed X.

10. TROUBLESHOOTING

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

1. Absence of positive signals in samples with Positive Control of Amplification (C+$_{Brucella}$) this may suggest that the programming of the temperature profile of the used Instrument was incorrect, or that the configuration of the PCR reaction was incorrect, or that the storage conditions for kit components did not comply with the manufacturer's instruction, or that the reagent kit expired. Programming of the used instrument (see 8.2.2.), storage conditions, and the expiration date of the reagents should be checked, and then PCR should be repeated for all samples.
2. If the Ct value of the sample in the JOE/Yellow channel greater than Z whereas the Ct value in the FAM/Green channel does not exceed X, PCR should be repeated. If in the second run the result is the same or the Ct value in the JOE/Yellow channel is less than Z, the result is considered to be **positive**.
3. If the Ct value is absent in both channels (JOE/Yellow and FAM/Green) or the Ct value in the FAM/Green channel is greater than X, PCR and detection should be repeated. If the result is

the same, it is necessary to repeat analysis of the sample starting from the DNA extraction stage.

4. If any signal is detected in the Negative Control of Extraction (C–) in the JOE/Yellow channel and in the Negative Control of Amplification (NCA) in any channel, this indicates contamination of reagents or samples. In this case, the results of the analysis of all samples are considered **invalid**. It is necessary to repeat the analysis of all samples and take measures to detect and eliminate the source of contamination.

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

11. TRANSPORTATION

AmpliSens® *Brucella* spp.-FRT 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.-FRT** PCR kit are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® *Brucella* spp.-FRT** 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.-FRT** PCR kit is no less than $1x10^3$ bacterial cells per 1 ml of sample.

The claimed analytical features of **AmpliSens® *Brucella* spp.-FRT** 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.-FRT** 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 **AmpliSens® *Brucella* spp.-FRT** 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, and 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.-FRT** 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+**	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
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
	Text	Positive Control STI (CS+) was changed to Positive Control STI-88 (CS+)
		Positive Control DNA *Brucella* (C+) was changed to Positive Control DNA *Brucella* ($C+_{Brucella}$)
21.06.11 LA	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”