



For *in Vitro* Diagnostic Use

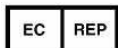
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AmpliSens® *Brucella* spp.-FRT PCR kit

Instruction Manual

AmpliSens®



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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 *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 real-time hybridization-fluorescence detection.

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 real-time 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. The real-time PCR 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, which contain 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.*-FRT** 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.*-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 <i>Brucella spp.</i> 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 <i>Brucella</i> (C+)	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, 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 during the DNA isolation 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 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, Rotor-Gene™ 3000 or Rotor-Gene™ 6000 (Corbett Research, Australia).
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity (for example, “Axygen”, USA).
- Refrigerator for temperature between 2 and 8 °C.
- Deep-freezer with temperature not more than minus16°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.*-FRT 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 (paraaortic, 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 is **25 µl**, volume of DNA sample is **10 µl**.

7.2.1. 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 into prepared tubes.
4. Carry 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 *Brucella*** to the tube labeled C+ (Positive Control of Amplification).

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

7.2.2. Amplification.

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

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

3. Fluorescence detection is on the 2-nd pass (**56 °C**) in FAM/Green and JOE/Yellow fluorometer channels.

4. Make the adjustment of the fluorescence channel sensitivity according to Appendix 1.

8. DATA ANALYSIS.

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

See Appendix 1 for data analysis settings for Rotor-Gene™ 3000 or Rotor-Gene™ 6000.

8.1. Results interpretation.

The results are interpreted by the software of Rotor-Gene™ 3000 or Rotor-Gene™ 6000 Instrument by the crossing (or not) 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 isolation	Pos (< X*)	Neg	OK
NCA	Amplification	Neg	Neg	OK
C+	Amplification	Neg	Pos (< Z*)	OK
CS+	Amplification	Pos (< Y*)	Neg	OK

*For X, Y, Z values see Appendix 1.

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

9. TROUBLESHOOTING.

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

1. Absence of positive signals in samples with 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.
2. If Ct value of the sample in JOE/Yellow channel more than Z, while Ct value in FAM/Green channel does not exceed X, PCR should be repeated. If in the second run the result is the same or Ct value in JOE/Yellow channel is less than Z the result is considered to be positive.
3. If Ct value is absent in both channels (JOE/Yellow and FAM/Green) or Ct value in FAM/Green channel is more than X, PCR and detection are to be repeated. If result is the same, it is required to repeat the sample analysis from the extraction stage.
4. If any signal is detected in Negative Control of Extraction (C-) in JOE/Yellow channel and in Negative

Control of Amplification (NCA) in any of channels, it indicates contamination of reagents or samples.

In this case results of the analysis for all samples are considered invalid. It is required 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 Authorised representative in the European Community.

10. STABILITY AND STORAGE.

All components of the **AmpliSens® Brucella spp.-FRT** 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.-FRT** PCR kit are to be stable until labeled expiration date.

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens® Brucella spp.-FRT** PCR kit is no less than 1×10^3 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 State Institution of Science Central Research Institute of Epidemiology) is used.

11.2. Specificity.

Specificity of **AmpliSens® Brucella spp.-FRT** 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.-FRT** 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.-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

14. EXPLANATION OF SYMBOLS.

	Manufacturer		Temperature limitation
	Use by		Batch code
	For <i>in Vitro</i> Diagnostic Use		Version
	Catalogue number		Authorised representative in the European Community.
	Contains sufficient for <n> tests		Caution, consult accompanying documents
	Consult instructions for use		Negative Control of Amplification
	Positive Control of Amplification		Negative control of Extraction
	Positive Control STI		Internal Control