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For Professional Use Only

BC-FRT

PCR kit

Instruction Manual

AmpliSens®

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1. INTENDED USE

BC-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Brucella* microorganisms DNA in the biological material by using real-time hybridization-fluorescence detection.

2. PRINCIPLE OF PCR DETECTION

Brucella detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen DNA specific region using special *Brucella* primers. After 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. **BC-FRT** PCR kit is a qualitative test, which contains the Internal Control (IC), the fag λ DNA fragment. 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. **BC-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 ºC.

3. CONTENT

BC-FRT PCR kit is produced in 2 forms:

BC-FRT PCR kit variant FRT (0.2-ml tubes) **REF** R-B69(RG)-CE;

BC-FRT PCR kit variant FRT (0.2-ml tubes) **REF** R-B69(iQ)-CE.

BC-FRT PCR kit variant FRT 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
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 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 protocol).

BC-FRT PCR kit variant FRT 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); iQ5 or iCycler iQ (Bio-Rad, USA) or equivalent).
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity (for example, Axygen, USA).
- Refrigerator with temperature between 2 and 8 °C.
- Deep-freezer with temperature no 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 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.
- Observe the rules and instructions to prevent semination of the environment objects.

Some components of this kit contain Sodium Azide as a preservative. Do not use metal tubing for reagent transfer.

6. SAMPLING AND HANDLING

BC-FRT PCR kit is intended for analysis of *Brucella* DNA *(B*. *melitensis*, *B*. *abortus*, *B*. *suis*, *B*. *ovis*, *B*. *canis)* extracted with DNA isolation kits from the biological material of animals:

- *stomach, spleen, liver contents* of abortus fetus*;*
- *placenta and fetus membranes;*
- *hygroma and bursa contents;*
- *blood and milk* of abortus animals or agglutinating serum or complement-fixing antibodies serum.

6.1. Material sampling

- Blood with 3 % EDTA (10:1).
- Other liquids – 10-15 ml.
- The parts of tissues and organs – about 1x1x1 (sm) or less.
- Whole lymph nodes.

In the case of killing animal use whole lymph nodes matched samples (paraaortic, over-udder, inguinal, pelvic) and parts of parenchymatous organ. Use testicles with epididymis of males with epididymitis or orchitis symptoms.

Materials are to be analyzed the next day after sampling if they stored at the temperature 2-8 °C.

Materials are to be stored at the temperature no more than minus 16 °C for one month.

6.2. Preparation of the samples

Whole blood samples with EDTA, synovial fluid, lymph nodes punctuates, hygroma and bursa contents and *microorganisms cell cultures* are used for DNA extraction without treatment.

Other liquid samples are centrifuged for 10-15 min at 3000 rpm. Carefully remove and discard the supernatant using a tip with aerosol barrier and leaving about 200 µl of the liquid on the sediment. If the sediment is hidden, transfer material again (10 ml) and repeat centrifugation. Suspend the sediment in supernatant and use 100 µl for DNA extraction.

If the liquid samples volume is not enough add saline to get 10 ml.

Parenchymatous organs, placenta, fetus membranes, testicles and *whole lymph nodes* pestle in sterile porcelain mortars or glass homogenizer, add equal volume of saline (1 ml) and thoroughly homogenize. Keep 30 min. Transfer 100 µl of the upper part of prepared suspension in sterile tube for DNA extraction.

7. WORKING CONDITIONS

BC-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA Isolation

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

- "DNA-sorb-B", **REF** K1-2-50-CE

Carry out the amplification according to the manufacturer instruction.
The volume of clinical sample is 100 µl.

8.2. Preparing the PCR

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

8.2.1. Preparing tubes for PCR

Variant FRT

1. Prepare the required number of the 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* (C+$_{Brucella}$)** to the tube labeled C+ (Positive control of Amplification).

IC+ -Add **10 µl** of **Positive Control STI-88 (CS+)** to the tube labeled IC+ (Positive control of IC Amplification).

8.2.2. Amplification

8.2.2.1. RG

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:

RG program for *Brucella*

Step	***Temperature, °C***	***Time***	***Fluorescence detection***	***Cycle repeats***
Hold	95	5 min	–	1
Cycling	95	10 s	–	10
	65	25 s	–	
	72	10 s	–	
Cycling 2	95	10 s	–	35
	56	25 s	detection	
	72	10 s	–	

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.

8.2.2.2. iQ

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

iQ program for *Brucella*

Step	*Temperature, °C*	*Time*	*Fluorescence detection*	*Cycle repeats*
Hold	95	5 min	–	1
Cycling	95	10 s	–	10
	65	25 s	–	
	72	25 s	–	
Cycling 2	95	10 s	–	35
	56	25 s	detection	
	72	25 s	–	

3. Fluorescence detection is on the 2-nd pass **(56 °C)** in FAM and HEX fluorometer channels.
4. Make the adjustment of the fluorescence channel sensitivity according to Appendix 2.

9. DATA ANALYSIS

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

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

iQ. Internal Control is detected in FAM, *Brucella* DNA is detected in JOE fluorescence channel.

See **Appendix 2** for data analysis settings for iQ5 or iCycler iQ.

Results interpretation

The results are interpreted by the software of Rotor-Gene 3000 or Rotor-Gene 6000 or iQ5 or iCycler iQ 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/HEX	Interpretation
C-	DNA isolation	Pos (< X*)	Neg	OK
NCA	Amplification	Neg	Neg	OK
C+	Amplification	Neg	Pos (< Y*)	OK

*For X, Y values see Appendix 1 in case of using Rotor-Gene 3000 or Rotor-Gene 6000 Instrument or Appendix 2 in case of using iQ5 or iQiCycler Instrument.

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

Results are accepted as relevant if both positive and negative controls of amplification along with negative control of extraction are passed.

10. TROUBLESHOOTING

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

1. If the Ct value is absent in both JOE/Yellow / HEX and FAM/Green channels or the Ct value in FAM/Green channel is higher than X, PCR reaction should be repeated. If the same result is achieved, the sample extraction process should be repeated.
2. If the Ct value is present for the Negative control of Extraction (C-) in JOE/Yellow / HEX channel and/or for Negative control of Amplification in FAM/Green and JOE/Yellow / HEX channels in the results grid, it indicates the contamination of reagent or samples. In such cases results of analysis must be considered as irrelevant. Test analysis must be repeated and measures to detect and eliminate the source of contamination are to be taken.
3. If no positive result is registered for Positive controls of Amplification, it can suggest incorrect programming of the temperature profile of used Instrument, incorrect configuration of the PCR reaction or storage conditions for kit components has not complied with manufacturer instruction, or the reagents kit has expired. PCR should be repeated.
4. If the Ct value is higher than Y in JOE/Yellow / HEX channel and the Ct value is not higher than X in FAM/Green channel, PCR reaction should be repeated. If the same result is achieved, the sample is considered to be positive.

11. TRANSPORTATION

BC-FRT PCR kit should be transported at 2–8 °C for no longer than 5 days.

12. STABILITY AND STORAGE

All components of **BC-FRT** PCR kit are to be stored at the temperature 2-8 °C when not in use. All components of the PCR kit are to be stable until labeled expiration date. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

13. SPECIFICATIONS

13.1. Sensitivity

Analytical Sensitivity of **BC-FRT** PCR kit is no less than $1x10^3$ copies per 1 ml of a sample (copies/ml).

The claimed analytical features of **BC-FRT** PCR kit are guaranteed only when additional kit of reagents “DNA-sorb-B” (manufactured by Federal State Institute of Science Central Research Institute of Epidemiology) is used.

13.2. Specificity

Specificity of **BC-FRT** PCR kit is ensured by selection of specific primers and strict reaction conditions as well as laboratory and clinical trials.

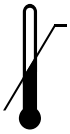
14. REFERENCES

1. Detection of *Brucella* species DNA in the stomach content of aborted sheep fetuses by PCR. B. Çetinkaya, H. Öngör, A. Muz, H. B. Ertas, H. Kalender, and H. M. Erdogan. Vet Rec., Vol. 144, Issue 9, 239-240, February 27, 1999.

15. QUALITY CONTROL

In compliance with Federal State Institute of Science Central Research Institute of Epidemiology ISO 13485 – certified Total Quality Management System, each lot of **BC-FRT** PCR kit is tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Sufficient for
RUO	Research Use Only		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation		Keep away from sunlight
	Upper limit of temperature	**NCA**	Negative control of amplification
	Manufacturer	**C–**	Negative control of extraction
	Date of manufacture	**C+**	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
17.04.11 LA	Text	The name of the PCR kit was changed from BRU-COM-FRT to BC-FRT
	Page footer	List Numbers of the PCR kit were changed from VET-9-FRT(RG)-K2-E and VET-9-FRT(iQ)-K2-E to R-B69(RG)-CE and R-B69(iQ)-CE
	Cover page	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 open reagents was added
	Key to Symbols Used	The explanation of symbols was corrected
27.05.13 PE	Cover page, text	Federal State Institute of Science “Central Research Institute for Epidemiology” was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
	Text	The name of the reagent “Positive Control DNA *Brucella*” was changed to “Positive Control DNA *Brucella* (C+$_{Brucella}$)”
	Key to Symbols Used	The explanation of symbols was corrected
14.06.13 PE	Cover page	Symbol IVD was changed to RUO
	Key to Symbols Used	
	Text	The name of the reagent “Positive Control STI-88” was changed to “Positive Control STI-88 (CS+)”