REF TVET9-50FRT

Bru-Com Real-TM

Real Time Kit for use with Rotor-Gene™ 2000/3000/6000 (Corbett Research, Qiagen)

Key to symbols used

REF	List Number	8°C 2°C	Store at 2-8°C
VET	For *Veterinary* Use		Caution!
LOT	Lot Number	VER	Version
	Expiration Date		Consult instructions for use
Σ	Contains reagents		Manufacturer

NAME
Bru-Com Real-TM

INTENDED USE
The **Bru-Com Real-TM** is a "Real-Time Amplification" test for the qualitative detection of *Brucella species* (B.melitensis, B.abortus, B.suis, B.ovis, B.canis) in the biological materials (whole blood, milk, tissue,etc) in the Rotor-Gene™ 2000/3000/6000/Q. Test contains an Internal Control (IC) which serves as an amplification control for each individually processed specimen and to identify possible reaction inhibition.

MATERIALS PROVIDED
Part N° 1 – **"DNA-Sorb-B":** isolation of DNA from specimens;
Part N° 2– **"Bru-Com Real-TM":** Real Time Amplification;

Part N° 1 – **"DNA-Sorb-B":**
- Lysis Solution, 15 ml;
- Washing Solution 1, 15 ml;
- Washing Solution 2, 50 ml;
- Sorbent, 1,25 ml;
- DNA-eluent, 5,0 ml.

Contains reagents for 50 extractions

Part N° 2– **"Bru-Com Real-TM":**
- **PCR-mix-1 *Brucella***, 55 ready-to-use single-dose test tubes;
- **PCR-mix-2-Flu**, 0,77 ml;
- ***Brucella* C+,** 0,1 ml;
- **Pos IC C**+, 0,1 ml ;
- **Negative Control C-, 1,2 ml**;
- **IC (Internal Control),** 0,5 ml;
- **DNA-buffer**, 0,5 ml;

Contains reagents for 55 tests.

MATERIALS REQUIRED BUT NOT PROVIDED
Zone 1: sample preparation:
- Biological cabinet
- Desktop microcentrifuge for "eppendorf" type tubes (RCF max. 16,000 x g); Eppendorf 5415D or equivalent
- 60°C ± 2°C dry heat block
- Vortex mixer
- Pipettors (capacity 5-40 µl; 40-200 µl; 200-1000 µl) with aerosol barrier
- 1,5 ml polypropylene sterile tubes (Sarstedt, QSP, Eppendorf)
- Disposable gloves, powderless
- Biohazard waste container

Zone 2: RT and amplification:

- Real Time Thermalcycler Rotor-Gene™ 2000/3000/6000/Q
- Workstation
- Pipettors (capacity 0,5-10 µl; 5-40 µl) with aerosol barrier
- Tube racks

WARNINGS AND PRECAUTIONS

1.  Lysis Solution contains guanidine thiocyanate. Guanidine thiocyanate is harmful if inhaled, or comes into contact with skin or if swallowed. Contact with acid releases toxic gas. (Xn; R: 20/21/22-36/37/38; S: 36/37/39).
2. Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterward.
3. Do not pipette by mouth.
4. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
5. Do not use a kit after its expiration date.
6. Dispose of all specimens and unused reagents in accordance with local regulations.
7. Biosafety Level 2 should be used for materials that contain or are suspected of containing infectious agents.
8. Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0,5% sodium hypochlorite, or other suitable disinfectant.
9. Avoid contact of specimens and reagents with the skin, eyes and mucous membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately.
10. Material Safety Data Sheets (MSDS) are available on request.
11. Use of this product should be limited to personnel trained in the techniques of amplification.
12. Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where you performed previous step.

STORAGE INSTRUCTIONS

Bru-Com Real-TM must be stored at 2-8°C.

STABILITY

Bru-Com Real-TM Test is stable up to the expiration date indicated on the kit label.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

Bru-Com Real-TM can analyze DNA extracted with **DNA-Sorb-B** from:

- *whole blood* collected in either ACD or EDTA tubes;
- *tissue* (≈1,0 gr) homogenized with mechanical homogenizer or scalpel, glass sticks, teflon pestles and dissolved in 1,0 ml of saline water or PBS sterile. Vortex vigorously and incubate 30 min at room temperature. Transfer the supernatant into a new 1,5 ml tube;
- *sinovial liquid;*
- *milk:* centrifuge 10 ml at 3000g/min for 10 min. Discard the supernatant and leave about 100 µl of solution for DNA extraction;

Specimens can be stored at +2-8°C for no longer than 12 hours, or freeze at -20°C to -80°C.
Transportation of clinical specimens and materials that contain or are suspected of containing infectious agents must comply with country, federal, state and local regulations for the transport of etiologic agents.

SPECIMEN AND REAGENT PREPARATION

1. **Lysis Solution** and **Washing Solution** (in case of their storage at +2-8°C) should be warmed up to 56°C until disappearance of ice crystals.
2. Prepare required quantity of 1.5 ml polypropylene tubes.
3. Add to each tube **10 µl** of **Internal Control** and **300 µl** of **Lysis Solution**.
4. Add **100 µl** of **Samples** to the appropriate tube.
5. Prepare Controls as follows:
   - add **100 µl** of **C–** (**Negative Control**) to labeled C*neg*.
6. Vortex the tubes, incubate for 5 min at 65°C and centrifuge for 3-5 sec.
7. Vortex vigorously **Sorbent** and add **25 µl** to each tube.
8. Vortex for 5-7 sec and incubate all tubes for 10 min at room temperature.
9. Centrifuge all tubes for 1 min at 10000g and using a micropipette with a plugged aerosol barrier tip, carefully remove and discard supernatant from each tube without disturbing the pellet. Change tips between tubes.
10. Add **300 µl** of **Washing Solution 1** to each tube. Vortex vigorously and centrifuge for 1 min at 10000g and using a micropipette with a plugged aerosol barrier tip, carefully remove and discard supernatant from each tube without disturbing the pellet. Change tips between tubes.
11. Add **500 µl** of **Washing Solution 2** to each tube. Vortex vigorously and centrifuge for 1 min at 10000g and using a micropipette with a plugged aerosol barrier tip, carefully remove and discard supernatant from each tube without disturbing the pellet. Change tips between tubes.
12. Repeat step 11.
13. Incubate all tubes with open cap for 5 min at 65°C.
16. Resuspend the pellet in **50 µl** of **DNA-eluent.** Incubate for 10 min at 65°C and vortex periodically.
17. Centrifuge the tubes for 2 min at maximum speed (12000-16000 g). The supernatant contains DNA ready for amplification. The amplification can be performed on the same day of extraction.

PROTOCOL:

1. Prepare required quantity of **PCR-mix-1 Brucella** tubes for samples and controls.
2. Add **7 µl** of **PCR-mix-2 Flu** into each tube.
3. Add **10 µl** of **extracted DNA** sample to appropriate tube.
4. Prepare for each panel 3 controls:
   - add **10 µl** of **DNA-buffer** to the tube labeled Amplification Negative Control;
   - add **10 µl** of ***Brucella* C+** to the tube labeled C+Bru;
   - add **10 µl** of **Pos IC C+** to the tube labeled IC C+;

Real Time Amplification with Rotor-Gene 2000/3000/6000/Q

1. Close PCR-mix-1 tubes and transfer them into the Rotor-Gene 2000/3000/6000/Q.
2. Select *New Run* and *Dual Labeled Probe.* Select Rotor Type *36-Well Rotor* and *No Domed 0,2 ml Tubes* (*Locking ring attached* for RG6000) Click *Next.*
3. Reaction volume: 25 µl. Make sure that for RG6000 the window "***15 µL oil layer volume***" is selected.
4. Click *Edit profile* and program Rotor-Gene 2000/3000/6000/Q as follows:

1. Hold	95 °C - 5 min
2. Cycling	95 °C - 10 sec
	65 °C - 25 sec
	72 °C -10 sec
	Cycle repeats – 10 times.
3. Cycling2	95 °C - 10 sec
	56 °C - 25 sec - detection
	72 °C -10 sec
	Cycle repeats – 35 times.

fluorescence detection on the channels Fam (Green) and Joe (Yellow) on the 2-nd pass (56°C)

Make the adjustment of the fluorescence channel sensitivity: *Channel Setup →Calibrate (Gain Optimisation for RG6000) → Perform Calibration (Optimisation) Before 1-st Acquisition.* Indicate *Min Reading* **5**, *Max Reading* **10** for *Fam/Sybr (Green)* and *Joe (Yellow)* channels.. In the column *Tube position* program position of the tubes in the carousel of the Rotor-Gene 2000/3000/6000/Q (the 1st position must contains reaction tube with reagents). Close the window *Auto Gain Calibration Setup.*

RESULTS ANALYSIS:

1. The results are interpreted with the software of **Rotor-Gene 2000/3000/6000/Q** through the presence of crossing of fluorescence curve with the threshold line. Internal Control is detected on the FAM (Green) channel, Brucella on the JOE (Yellow) channel.
2. Press *Analysis* then select button *Quantitation*. Perform the operation for the channel Fam (*Cycling A FAM)*, then for the channel Joe (*Cycling A JOE)*
3. Select *Dynamic Tube, Threshold*: 0,1
4. In the table of results (*Quantitation Results*) appear the values of Ct (Threshold cycle). The Ct value of the samples and controls on the *Fam (Green)* channel should be ≤ 31.

Table 2. Results for controls

Control	Stage for control	Ct channel Fam (Green)	Ct channel Joe (Yellow)	Interpretation
NCS	DNA isolation	Pos (< 31)	Neg	Valid result
DNA-buffer	Amplification	Neg	Neg	Valid result
Brucella C+	Amplification	Neg	Pos (< 33)	Valid result
Pos IC C+	Amplification	Pos (< 31)	Neg	Valid result

5. Specimens with Ct ≤ 33 in the channel Joe (Yellow) (*Quant. Resultes – Cycling A. Joe)* are interpreted as positive for *Brucella.*
6. The sample is considered to be negative if in the channel Joe (Yellow) the Ct value is not determined (the fluorescence curve does not cross the threshold line) and in the results table on the channel Fam (Green) the Ct value is lower than 31.

Troubleshooting

1. Occurrence of any value Ct in the table of results for negative control of amplification (DNA-buffer) testifies contamination of reagents or samples. In this case results of the analysis for all tests are considered invalid. It is required to repeat the analysis of all tests, and also to take measures to detect and eliminate the source of contamination.
2. No signal with Brucella C+ on the Joe (Yellow) channel and with Pos IC C+ on the Fam (Green) channel indicates incorrect programming of the Real Time instrument: repeat the amplification with correct setting.

PERFORMANCE CHARACTERISTICS

Analytical specificity

The analytical specificity of the primers and probes was validated with negative samples. They did not generate any signal with the specific *Brucella* primers and probes. The specificity of the kit **Bru-Com Real-TM** was 100%. The potential cross-reactivity of the kit **Bru-Com Real-TM** was tested against the group control. It was not observed any cross-reactivity with other pathogens.

Analytical sensitivity

The kit **Bru-Com Real-TM** allows to detect *Brucella DNA* in 100% of the tests with a sensitivity of not less than 1000 copies/ml. The detection was carried out on the control standard and its dilutions by negative sample.

Target region: WboA gene

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