

GUIDELINES

to **AmpliSens[®] *Brucella* spp.-FRT PCR kit**

for qualitative detection of DNA of *Brucella* species (*B.melitensis*,
B.abortus, *B.suis*, *B.ovis*, *B.canis*, *B.neotomae*) in human and
animal biological materials and bacterial culture by the polymerase
chain reaction (PCR) with real-time hybridization-fluorescence
detection

AmpliSens[®]



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INTENDED USE

The guidelines describe the procedure of using **AmpliSens® *Brucella* spp.-FRT** PCR kit 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 the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection using the following instruments

- Rotor-Gene 3000, Rotor-Gene 6000 (Corbett Research, Australia).

AMPLIFICATION AND DATA ANALYSIS USING Rotor-Gene 3000/6000 (Corbett Research, Australia)

When working with Rotor-Gene 3000 one should use the Rotor-Gene version 6.1 and higher software and the Rotor-Gene 6000 versions 1.7 (build 67) software or higher for Rotor-Gene 6000 instrument.

Hereinafter, all terms corresponding to different instruments and software are indicated in the following order: for Rotor-Gene 3000/for Rotor-Gene 6000.

Programming the thermocycler

1. Click the **New** button in the software main menu.
2. In opened window select the **Advanced** tab and the **Dual Labeled Probe/Hydrolysis probes** template. Click the **New** button.
3. Select the **36-Well Rotor** and tick the **No Domed 0.2 ml Tubes/Locking ring attached** option.
4. Click the **Next** button.
5. Select the **Reaction volume** as **25 µl**. Tick the **15 µl oil layer volume** option.
6. Click the **Next** button.
7. Click the **Edit profile** button and set the amplification program:

Table 1

AmpliSens amplification program

Step	Temperature, °C	Time	Fluorescence detection	Cycles
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	FAM/Green, JOE/Yellow	
	72	10 s	–	

8. Click the **OK** button.

9. At the bottom of the window click the **Calibrate/Gain Optimisation...** button. In the opened window click the **Calibrate Acquiring/Optimise Acquiring** button. Indicate **Min Reading 5, Max Reading 10** for both channels. In the **Tube position** column specify the number of the test tube, on which the **Gain** parameter is automatically set. By default it is the first tube in the rotor. So the well No.1 should be always filled by the tube with the reaction mixture. Check with the checkmark **Perform Calibration Before 1st Acquisition/ Perform Optimisation Before 1st Acquisition**. Close the **Auto Gain Calibration Setup** window by clicking **Close** button. Click the **Next** button.
10. Place the prepared tubes in the rotor of the instrument. Start the amplification by the **Start run** button.
11. Name the experiment and save it to the disk (the results of the experiment will be automatically saved in this file).

Enter the order of the tubes in the rotor during amplification run or after the run is over. To do this, use the **Edit samples** button (at the right bottom corner of the main window). Indicate all samples and controls as **Unknown** in the **Samples** menu.

Data analysis

Data analysis of the IC amplification in the FAM/Green channel

1. Activate the **Analysis** button in the menu, select the mode of the analysis **Quantitation**, activate the **Cycling A. FAM/Cycling A. Green, Show** buttons.
2. Cancel the automatic choice of the threshold line level **Threshold**.
3. Select the **Linear scale** button at the right bottom corner of the window. If this button is enabled by default then the **Log scale** button will be seen.
4. Activate the **Dynamic tube** button in the menu of main window (**Quantitation analysis**).
5. Select the **More Settings/Outlier Removal** parameter and set **NTC threshold** value as **0 %**.
6. In **CT Calculation** menu (in the right part of the window) indicate the threshold line level **Threshold = 0.1**.
7. In the results grid (the **Quant. Results** window) one will be able to see the **Ct** values.

Data analysis of the Brucella DNA amplification in the JOE/Yellow channel

1. Activate the **Analysis** button in the menu, select the mode of the analysis **Quantitation**, activate the **Cycling A. JOE/Cycling A. Yellow, Show** buttons.
2. Cancel the automatic choice of the threshold line level **Threshold**.
3. Select the **Linear scale** button at the right bottom corner of the window. If this button is enabled by default then the **Log scale** button will be seen.

4. Activate the **Dynamic tube** button in the menu of main window (**Quantitation analysis**).
5. Select the **More Settings/Outlier Removal** parameter and set **NTC threshold** value as **10 %**.
6. In **CT Calculation** menu (in the right part of the window) indicate the threshold line level **Threshold = 0.1**.
7. In the results grid (the **Quant. Results** window) one will be able to see the **Ct** values.

Table 1

Boundary Ct values		
Sample	Channel	Ct value
C-	FAM/Green	31
C+Brucella	JOE/Yellow	33
CS+	FAM/Green	31
Test samples	FAM/Green	31
	JOE/Yellow	33