

## GUIDELINES

to **AmpliSens<sup>®</sup> *Bacillus anthracis*-FRT** PCR kit for qualitative detection of DNA of vegetative and cryptogamic forms of *Bacillus anthracis* in the biological material and environmental samples and for determination of *Bacillus anthracis* plasmid composition by identification of *pagA* (plasmid pXO1) and *capA* (plasmid pXO2) genes by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection

### AmpliSens<sup>®</sup>



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## TABLE OF CONTENTS

INTENDED USE .....	2
AMPLIFICATION AND DATA ANALYSIS USING Rotor-Gene 3000/6000 (Corbett Research, Australia) .....	3

## INTENDED USE

The guidelines describe the procedure of using **AmpliSens® *Bacillus anthracis*-FRT** PCR kit for qualitative detection of DNA of vegetative and cryptogamic forms of *Bacillus anthracis* in biological material and environmental samples and for determination of *Bacillus anthracis* plasmid composition by identification of *pagA* (plasmid pXO1) and *capA* (plasmid pXO2) genes 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 the terms corresponding to different instruments and software are indicated in the following order: for Rotor-Gene 3000 / for Rotor-Gene 6000.**

Carry out the sample pretreatment and reaction mixture preparation stages according to the PCR kit *Instruction Manual*.

### 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** option.
4. Click the **Next** button.
5. Select the **Reaction volume - 25 µl**. Tick the **15 µl oil layer volume** option.
6. Click the **Next** button.
7. Click the **Edit profile** button.
8. Set the amplification program.

Table 1

**AmpliSens amplification program**

<b>Step</b>	<b>Temperature, °C</b>	<b>Time</b>	<b>Fluorescence detection</b>	<b>Cycles</b>
Hold	95	5 min	–	1
Cycling	95	10 s	–	10
	60	25 s	–	
	72	10 s	–	
Cycling 2	95	10 s	–	35
	56	25 s	FAM/Green, JOE/Yellow, ROX/Orange	
	72	10 s	–	

9. After setting up the temperature profile click twice the **OK** button
10. At the bottom of the window click the **Calibrate/Gain Optimization** button. In the opened window click the **Calibrate Acquiring/Optimise Acquiring** button. Set the following parameters:
  - **FAM/Green channel: Min Reading – 20 FI, Max Reading – 30 FI;**
  - **JOE/Yellow channel: Min Reading – 10 FI, Max Reading – 15 FI;**
  - **ROX/Orange channel: Min Reading – 5 FI, Max Reading – 10 FI.**

**Gain** parameter will be set automatically to the tube defined in the **Tube position** column. 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. Select the **Perform Calibration Before 1<sup>st</sup> Acquisition/Perform Optimisation Before 1<sup>st</sup> Acquisition** option. Click the **Close** and then the **Next** buttons.
11. Place the prepared tubes in the rotor of the instrument. Start the amplification by the **Start run** button.
12. 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 the amplification run or by the end of it. To do it select the **Edit samples** button. Indicate all samples and controls as **Unknown** in the **Samples** menu.

### Data analysis

#### Data analysis of the IC amplification in the ROX/Orange channel

1. Activate the **Analysis** button in the menu, select the mode of the analysis **Quantitation**, activate the **Cycling A. ROX** or **Cycling A. Orange, Show** buttons.
2. Cancel the automatic choice of the threshold line level (**Threshold**).
3. Select the **Linear Scale** button at the bottom of the window (if linear scale is turned on 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. In the **CT Calculation** menu (in the right part of the window) indicate the threshold line level **Threshold = 0.1**.
6. In the results grid (the **Quant. Results** window) one will be able to see the *Ct* values

#### Data analysis of *Bacillus anthracis* pXO1 DNA 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** or **Cycling A. Green, Show** buttons.

2. Cancel the automatic choice of the threshold line level (**Threshold**).
3. Select the **Linear Scale** button at the bottom of the window (if linear scale 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. In **CT Calculation** menu (in the right part of the window) indicate the threshold line level **Threshold = 0.025**.
6. In the results grid (the **Quant. Results** window) one will be able to see the *Ct* values.

Data analysis of *Bacillus anthracis* pXO2 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** or **Cycling A. Yellow, Show** buttons.
2. Cancel the automatic choice of the threshold line level (**Threshold**).
3. Select the **Linear Scale** button at the bottom of the window (if linear scale is turned on 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. In **CT Calculation** menu (in the right part of the window) indicate the threshold line level **Threshold = 0.1**.
6. In the results grid (the **Quant. Results** window) one will be able to see the *Ct* values.

Table 2

**Boundary *Ct* values**

Sample	Channel	<i>Ct</i> value
<b>C-</b>	ROX/Orange	31
<b>C+</b> <i>Bacillus anthracis</i> pXO1	FAM/Green	33
<b>C+</b> <i>Bacillus anthracis</i> pXO2	JOE/Yellow	33
<b>CS+</b>	ROX/Orange	31
<b>Test samples</b>	FAM/Green	33
	JOE/Yellow	33
	ROX/Orange	31