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GUIDELINES

to AmpliSens[®] TBE-FRT PCR kit

for qualitative detection of *tick-borne encephalitis virus* RNA in the biological material 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® TBE-FRT** PCR kit for qualitative detection of *tick-borne encephalitis virus* RNA in the biological material (blood plasma and serum; leucocytic fraction of blood; cerebrospinal fluid; autopsy material of human and animal (brain tissue); ticks) by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection using Rotor-Gene 3000/6000 (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*. When carrying out the amplification it is recommended to use thin-walled PCR tubes (0.2 ml) with flat caps (e.g. Axygen, USA), or PCR tubes (0.1 ml) with caps from the four-pieces-strips (e.g. Corbett Research, Australia; QIAGEN, Germany) (detection through the bottom of the tube).

Programming the thermocycler

1. Turn on the instrument, run the Rotor-Gene software.
2. Insert the tubes or strips into the rotor of the Rotor-Gene 3000/6000 instrument beginning from the first well (the rotor wells are numbered, the numbers are used for the further programming of the samples' order in the thermocycler). Insert the rotor into the instrument, close the lid.

NOTE: Well 1 must be filled with any test tube.

3. Program the instrument according to the *Instruction Manual* given by the manufacturer of the instrument.

Creating the template for the run

1. Set the amplification program.

Table 1

Amplification program

Step	Temperature, °C	Time	Fluorescence detection	Cycles
Hold	50	30 min	-	1
Hold 2	95	15 min	-	1
Cycling	95	10 s	-	5
	65	45 s	-	
	72	15 s	-	
Cycling 2	95	10 s	-	45
	60	45 s	FAM/Green, JOE/Yellow	
	72	15 s	-	

- Click the **Calibrate/Gain Optimisation...** button in the **New Run Wizard** window. In the opened window:
 - for calibration in FAM/Green, JOE/Yellow channels select the **Calibrate Acquiring/Optimise Acquiring** button;
 - perform the calibration in the selected channels before the first detection (tick the **Perform Calibration Before 1st Acquisition/Perform Optimisation Before 1st Acquisition** option);
 - set calibration for the FAM/Green channel: from **5FI** to **10FI**, for the JOE/Yellow channel from **5FI** to **10FI** (activate the **Auto Gain Calibration Setup** window). Click the **Close** button
- Start the amplification by the **Start run** button. Name the experiment and save it to the disk (the results of the run will be automatically saved in this file).
- Enter the data into the grid of the samples (it opens automatically after the amplification has been started). Enter the names/numbers of the test samples in the **Name** column. Define the Negative Control of amplification as NCA, the Positive Control of amplification as C+. Set the type **Unknown** opposite all the test samples, the type **Positive control** – the Positive control of amplification, the type **Negative Control**– for the Negative control of amplification. Set the type **None** for the cells matching with the corresponding empty tubes.

Data analysis

The obtained results are analyzed by the Rotor-Gene software. The results are interpreted according to the crossing (or not-crossing) of the S-shaped (sigmoid) fluorescence curve with the threshold line set at the specific level, that corresponds to the presence (or absence) of the *Ct* (threshold cycle) value of the DNA sample in the corresponding column of the results table.

Data analysis of TBE RNA (FAM/Green channel)

1. Click the **Analysis** button in the menu, select the mode of the analysis **Quantitation**, activate the buttons **Cycling A. FAM/Cycling A. Green, Show**.
2. Cancel the automatic choice of the threshold line level **Threshold**.
3. Activate the **Dynamic tube** and **Slope Correct** buttons in the menu of main window (**Quantitation analysis**).
4. In the **CT Calculation** menu (in the right part of the window), indicate the threshold line level **0.03** in the **Threshold** box and set **10 %** for the value **More settings/Outlier Removal**.
5. In the results grid (the **Quantitation Results** window) one will be able to see the *Ct* values.

Data analysis of the IC amplification (JOE/Yellow channel)

1. Click the **Analysis** button in the menu, select the mode of the analysis **Quantitation**, activate the buttons **Cycling A. JOE / Cycling A. Yellow, Show**.
2. Cancel the automatic choice of the threshold line level **Threshold**.
3. Activate the **Dynamic tube** and **Slope Correct** buttons in the menu of main window (**Quantitation analysis**).
4. In the **CT Calculation** menu (in the right part of the window), indicate the threshold line level **0.03** in the **Threshold** box and set **15 %** for the value **More settings/Outlier Removal**.
5. In the results grid (the **Quantitation Results** window) one will be able to see the *Ct* values.

Table 2

Boundary Ct values

Sample	Channel	Ct value
C-	JOE/Yellow	28
PCE	FAM/Green	37
	JOE/Yellow	28
C+TBE	FAM/Green	29
Test samples	FAM/Green	37
	JOE/Yellow	28