

IVD

For Professional Use Only

## GUIDELINES

**to AmpliSens<sup>®</sup> *Rotavirus* / *Norovirus* / *Astrovirus-FRT* PCR kit**  
for qualitative detection and differentiation of *Rotavirus A*, *Norovirus*  
genotype 2, and *Astrovirus* RNA in environmental samples and  
clinical material by the polymerase chain reaction (PCR) with  
real-time hybridization-fluorescence detection

# AmpliSens<sup>®</sup>



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## INTENDED USE

The guidelines describe the procedure of using **AmpliSens® Rotavirus / Norovirus / Astrovirus-FRT** PCR kit for detection and differentiation of *Rotavirus A*, *Norovirus* genotype 2, and *Astrovirus* RNA in the clinical material and environmental samples 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),
- Rotor-Gene Q (QIAGEN, Germany),
- iCycler iQ5 (Bio-Rad, USA).
- CFX96 (Bio-Rad, USA);

## AMPLIFICATION AND DATA ANALYSIS USING Rotor-Gene 3000/6000 (Corbett Research, Australia), Rotor-Gene Q (QIAGEN, Germany) INSTRUMENTS

When working with Rotor-Gene 3000 one should use the Rotor-Gene Version 6 software and the Rotor-Gene 6000 versions 1.7 (build 67) software or higher for Rotor-Gene 6000 and Rotor-Gene Q instruments.

**Hereinafter, all 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 Rotor-Gene 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. Switch on the Rotor-Gene 3000/6000/Q instrument.
2. Insert the tubes into the carousel of the Rotor-Gene 3000/6000/Q instrument (the carousel cells are numbered, the numbers are used for the further programming of the samples' position in the thermocycler). Program the instrument.

**NOTE:** Well No. 1 must be filled with a test tube. The tube with RT-PCR-mix-1-FEP/FRT *Rotavirus / Astrovirus* must be placed in the first well if tubes with several reaction mixture types are loaded in the rotor simultaneously

3. Click **New** in the main program menu.
4. Choose the reaction mixture volume and the rotor type:

**Reaction volume–25 µl**

**Rotor type–36-Well Rotor or 72-Well Rotor** depending on the rotor type.

5. Set the amplification program by pressing the **Edit profile** button (see Table 1).

Table 1

**The amplification program for rotor-type instruments**

<b>Step</b>	<b>Temperature, °C</b>	<b>Time</b>	<b>Repeats</b>
1	50	30 min	1
2	95	15 min	1
3	95	10 s	45
	60	25 s <i>fluorescent signal detection</i>	
	72	10 s	

Detection of fluorescent signal is enabled in the FAM/Green and JOE/Yellow channels (detection is enabled in other channels if several tests are carried out simultaneously).

6. Set calibration parameters (activate **Calibrate/Gain Optimisation...** in a new experiment module):
- Perform fluorescence measurement in FAM/Green and JOE/Yellow channels (activate **Calibrate Acquiring/Optimise Acquiring**);
  - Perform calibration before the first measurement in FAM/Green and JOE/Yellow channels (activate **Perform Calibration Before 1<sup>st</sup> Acquisition/Perform Optimisation Before 1<sup>st</sup> Acquisition**);
  - Set channel calibrations: for FAM/Green channel by the first tube and from 5FI to 10FI; for JOE/Yellow channel, from 5 FI to 10FI (activate the **Edit...** button, **Auto gain calibration channel settings** window);
7. Select the **Start run** button for amplification run and name the experiment.
8. During or after amplification, it is necessary to program the placement of test samples, negative control of extraction, positive and negative controls of RNA reverse transcription and cDNA amplification. Enter the data in the table of samples (opens automatically after the thermocycling process starts). Indicate the names/numbers of test samples in the **Name** column. Indicate Positive Control of RT-PCR as **C+**, Negative Control of RT-PCR as **C-**. Set the **Unknown** type next to all test samples, the **Positive Control** type next to the Positive Controls, the **Negative Control** type next to the Negative Control of Extraction, and the **NTC** type next to the Negative Control of RT-PCR. For empty wells, indicate **None**.

### Data analysis

1. Press the **Analysis** button, then select the **Quantitation** analysis mode and press the **Cycling A. JOE/Cycling A. Yellow, Show** and **Cycling A. FAM/Cycling A. Green, Show** buttons.
2. Cancel the **Threshold** automatic choice for each opened main window (FAM/Green

and JOE/Yellow).

3. Activate the **Dynamic tube** and **Slope Correct** buttons in the main window menu (**Quantitation analysis**).
4. In the **CT Calculation** menu (in the right part of window), set **Threshold = 0.05** and **More settings** as **5 - 10 %** (for FAM/Green channel) and **10%** (for JOE/Yellow channel).
5. In the results grid (**Quant. Results** window), *Ct* values for each test sample will appear.
6. *Ct* values for test samples are taken into account only if the results obtained for control samples C-, C+*Rotavirus / Astrovirus*, C+*Norovirus genotype 2 / ST1*, and NCA are correct according to *Ct* values specified in the *Important Product Information Bulletin* for the PCR kit.
7. Data for the test samples are analyzed separately for each type of RT-PCR-mix-1-FEP/FRT according to *Ct* values specified in *Important Product Information Bulletin* for the PCR kit.

## AMPLIFICATION AND DATA ANALYSIS USING iCycler iQ5 (Bio-Rad, USA)

### INSTRUMENT

Carry out the sample pretreatment and reaction mixture preparation stages according to the PCR kit *Instruction Manual*. When working with the iCycler iQ5 instrument, it is recommended to use 0.2-ml disposable tubes for PCR with domed caps (for example, Axygen, USA).

### Programming the iCycler iQ5 instrument (Bio-Rad, USA)

1. Switch on the instrument and optical module at least 20–30 min before measurement.
2. Click the **Create new** button in the **Workshop** module.
3. Set the amplification program (**Protocol**) (see Table 2).

Table 2

The amplification program for plate-type instruments

Step	Temperature, °C	Time	Repeats
1	50	30 min	1
2	95	15 min	1
3	95	10 s	45
	60	25 s fluorescent signal detection	
	72	10 s	

Detection of fluorescent signal is enabled in the FAM and JOE/HEX channels (detection is enabled in other channels if several tests are carried out simultaneously).

Name and save the new protocol.

4. Set the order of sample placement in the plate (**Plate**), select channels for fluorophores FAM and HEX (**Select/add fluorophores**), activate fluorophores for the samples in the created protocol by pressing the **Fluorophore loading in Whole Plate mode** button. Set **Sample Volume – 25 µl**, **Seal Type – Domed cap**, **Vessel Type – Tubes**. Save the created protocol by pressing the **Save/Exit Plate Editing** button.
5. Place the tubes in the instrument strictly in accordance with the created plate setup.
6. Press the **Run** button, select **Run Persistent Plate** and save the experiment.

### Data analysis

1. Data are analyzed in the channels for fluorophores HEX and FAM.
2. Activate the **Data Analysis** button in the menu window.
3. Select a range of 20–25 for **Base Line** in the **Crossing Threshold User Defined** menu. Normally, the threshold line should cross only the sigmoid curves of signal accumulation of positive samples and controls and should not cross curves of other shape. Otherwise, the threshold level should be raised.

4. Ct values for test samples are taken into account only if the results obtained for control samples C-, C+*Rotavirus / Astrovirus*, C+*Norovirus* genotype 2 / ST1, and NCA are correct (see the *Information Product Important Bulletin* for the PCR kit).
5. Results obtained for the test samples should be interpreted in accordance with the Instruction Manual and the *Important Product Information Bulletin*.

## AMPLIFICATION AND DATA ANALYSIS USING CFX96 (Bio-Rad, USA)

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 domed or optically transparent flat caps (detection through the cap of the tube).

**NOTE:** Monitor the tubes. There must not be drops left on the walls of the tubes as falling drops during the amplification process may lead to the signal failure and complicate the results analysis. Don't turn the strips upside down while inserting them into the instrument.

Program the instrument according to the *Instruction Manual* provided by the manufacturer.

### Programming the thermocycler

1. Turn on the instrument and start the **Bio-Rad CFX Manager** program.
2. Select **Create a new Run** (or select **New** and then **Run...** in the **File** menu).
3. In the **Run Setup** window, select **Protocol** and click the **Create new...** button. Set amplification parameters (time, temperature, cycles, and fluorescence acquiring cycle) in the opened **Protocol Editor – New** window (see Table 5). Set **Sample Volume – 25 µl**.

Table 3

The amplification program for plate-type instruments

Step	Temperature, °C	Time	Number of cycles
1	50	30 min	1
2	95	15 min	1
3	95	10 s	45
	60	25 s <i>fluorescent signal detection</i>	
	72	10 s	

Detection of fluorescent signal is enabled in the FAM and HEX channels (detection is enabled in other channels if several tests are carried out simultaneously).

**NOTE!** Set **Ramp Rate 2,5 °C/s** by clicking the **Step Options** button for each step of cycling.

4. In the **Protocol Editor New** window select **File**, then **Save As**, and name the protocol. This protocol can be used for further runs by clicking the **Select Existing...** button in the **Protocol** tab.  
When the required program is entered or edited, click **OK** at the bottom of the window.
5. In the **Plate** tab click the **Create new...** button. Set the tube order in the opened **Plate Editor – New** window. In the **Sample type** menu select **Unknown**; click the **Select Fluorophores...** button and indicate the required fluorophores with a checkmark; click **OK**; then indicate with a checkmark the fluorescence signal acquiring for the selected



wells in the required channels. Define sample names in the **Sample name** window.

**NOTE!** Detection is enabled in other channels if several tests are carried out simultaneously.

6. In the **Plate Editor New** window select **File**, then **Save As**, and name the plate. When the required plate is entered or edited, click **OK** at the bottom of the window.
7. Place the reaction tubes in the wells of the instrument in accordance with the entered plate setup. In the **Start Run** tab click the **Start Run** button then save the file of the experiment.
8. Proceed to the analysis of results after the end of the run.

### **Data analysis**

Obtained data are interpreted by the real-time PCR instrument software by the crossing of a fluorescence curve with the threshold line set at the specific level (that corresponds to the presence of *Ct* value in the results grid).

1. The fluorescence curves, plate setup, and results grid with *Ct* values are displayed in the **Quantification** tab.
2. For each channel at a time set the threshold line (drag it with a cursor while pressing the left mouse button) at the level of 10-20 % of maximum fluorescence obtained for the Positive Controls in the last amplification cycle. Make sure that fluorescence curve of the Positive Control crosses the threshold line at the zone of exponential growth of fluorescence passing onto linear growth.
3. Click the **View/Edit Plate** button on the toolbar and enter sample names in the opened window, if required.
4. The *Ct* values of test samples are taken into account only in case of if the results for the positive and negative controls of the amplification and the negative control of the RNA extraction are correct in accordance with the *Instruction Manual* and the *Important Product Information Bulletin* enclosed to the PCR kit.
5. The interpretation of test samples should be performed in accordance with the *Instruction Manual* and the *Important Product Information Bulletin* enclosed to the PCR kit. The samples are considered positive if their *Ct* level is not higher than the value of the threshold cycle specified in the *Important Product Information Bulletin*.


Click **Tools** on the toolbar, then **Reports...**, and then save the generated report.

## TROUBLESHOOTING

If the threshold value was defined in the results grid for the sample, but the fluorescence curve for this channel does not have the correct (S-shaped) form with an area of the typical exponential fluorescence growth (the curve is linear), the result is incorrect, it cannot be interpreted as positive. Such a result may suggest incorrect setting of the threshold line (or other analysis parameters). If the result is obtained with the correct threshold level (and other parameters), then the result is erroneous for this sample, it is interpreted as negative.

If the threshold value was defined in the results grid for the Negative Control, but the fluorescence curve for this channel does not have the correct (S-shaped) form with an area of the typical exponential fluorescence growth (in particular the curve is linear), the result is erroneous, it is interpreted as negative.

### List of Changes Made in the Guidelines

VER	Location of changes	Essence of changes
25.06.11 LA	Cover page	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
17.12.11 VV	Page footer	Reference number, <b>REF</b> R-V40(RG,iQ)-CE-B, was added
06.04.12 lvi	Amplification and data analysis using Rotor-Gene 3000/6000 (Corbett Research, Australia), Rotor-Gene Q (Qiagen, Germany)	The Rotor-Gene Q (Qiagen, Germany) instrument is added
		The amplification program and programming parameters are added
		The following phrase is added “The tube with RT-PCR-mix-1-FEP/FRT <i>Rotavirus</i> / <i>Astrovirus</i> must be placed in the first well if tubes with several reaction mixture types are loaded in the rotor simultaneously”
	Amplification and data analysis using iCycler iQ5 (Bio-Rad, USA)	The amplification program and programming parameters are added
		The information that tubes must be placed in the instrument strictly in accordance with the created plate setup is added.
16.09.13 FN	Footer	Catalogue number <b>REF</b> R-V40(RG,iQ)-CE-B was deleted
02.04.14 SA	Cover page	Address of European representative was added
27.10.14 PM	Intended Use	Information about the real-time instrument, CFX96 (Bio-Rad, USA), was added
	Amplification and data analysis using CFX96 (Bio-Rad, USA)	The section was added
23.09.15 PM	Amplification and data analysis using Rotor-Gene 3000/6000 (Corbett Research, Australia) and Rotor-Gene Q (QIAGEN, Germany) instruments	<i>More setting</i> parameter for FAM/Green channel was changed from <b>10%</b> to <b>5 - 10 %</b> (for FAM/Green channel) and <b>10%</b> (for JOE/Yellow channel)
22.03.19 EM	Troubleshooting	The section was added
25.12.20 EM	Through the text	The symbol  was changed to NOTE:
	Cover page	The phrase “Not for use in the Russian Federation” was added
11.03.21 MM	Front page	The name, address and contact information for Authorized representative in the European Community was changed