## **QIAsymphony<sup>®</sup> RGQ Protocol Sheet**

# Settings to run artus<sup>®</sup> QS-RGQ Kits (Rotor-Gene<sup>®</sup> Q software 2.1, or higher)

CE	artus BK Virus QS-RGQ Kit	Version 1, <b>REF</b> 4514363
<b>CE</b> 0197	artus CMV QS-RGQ Kit	Version 1, <b>REF</b> 4503363
CE	artus EBV QS-RGQ Kit	Version 1, <b>REF</b> 4501363
<b>CE</b> 0197	artus HBV QS-RGQ Kit	Version 1, <b>REF</b> 4506363, 4506366
<b>CE</b> 0197	artus HCV QS-RGQ Kit	Version 1, <b>REF</b> 4518363, 4518366
<b>CE</b> 0197	artus HI Virus-1 QS-RGQ Kit	Version 1, <b>REF</b> 4513363, 4513366
CE	artus HSV-1/2 QS-RGQ Kit	Version 1, <b>REF</b> 4500363
CE	artus VZV QS-RGQ Kit	Version 1, <b>REF</b> 4502363

#### Version management

This document is the QIAsymphony RGQ Protocol Sheet, Version 1, R3.



Check availability of new electronic labeling revisions at <u>www.qiagen.com</u> before test execution.

### Important points before starting

- Take time to familiarize yourself with the Rotor-Gene Q before starting the protocol. See the instrument user manual.
- See also the relevant artus QS-RGQ Kit handbook and Application Sheet at www.qiagen.com/products/qiasymphonyrgq.aspx.
- Make sure that at all quantitation standards as well as at least one negative control (Water, PCR grade) are included per PCR run. To generate a standard curve, use all the quantitation standards supplied.



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#### Procedure

- 1. Place the PCR tubes in the 72-Well Rotor of the Rotor-Gene Q. Seal the rotor with the locking ring.
- 2. Transfer the cycler file from the QIAsymphony AS to the Rotor-Gene Q computer.
- 3. Open the "New Run Wizard" dialog box (Figure 1). Check the "Locking Ring Attached" box and click "Next".

	Welcome to the Advanced R Rotor Type 36-Well Rotor 72-Well Rotor	un Wizard!	
	Gene-Disc 72 Gene-Disc 100		
¢ 0000 4018			
	Skip Wizard << <u>B</u> ack	Next >>	_

Figure 1. The "New Run Wizard" dialog box.

4. Select 50 for the PCR reaction volume and click "Next" (Figure 2).

New Run Wizard				<u>×</u>
00000000000000000000000000000000000000	clicking Next when you	scellaneous options for the run u are ready to move to the next gen		This box displays help on elements in the wizard. For help on an item, hover your mouse over the item for help. You can also click on a combo box to display help about its available settings.
	Reaction Volume (µL): Sample Layout : A1,	A2, A3,	T	
	Skip Wizard	< <u>B</u> ack <u>N</u> ext >>		,

Figure 2. Setting the general assay parameters. Note: Even if the physical reaction volume is not 50  $\mu$ l, make sure to select 50 for the reaction volume in the Rotor-Gene software.

QIAsymphony RGQ Protocol Sheet: Settings to run artus QS-RGQ Kits (Rotor-Gene Q software 2.1, or higher)page 2 of 7

5. Click the "Edit Profile" button in the next "New Run Wizard" dialog box (Figure 3). Program the appropriate temperature profile for the relevant artus QS-RGQ Kit as shown in Table 1, using the example screenshots in Figures 3–6 as a guide (screenshots for the artus HI Virus-1 QS-RGQ Kit are shown as examples).

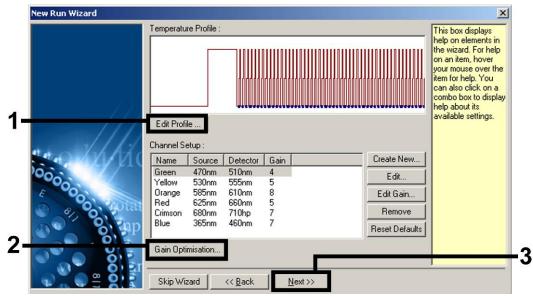
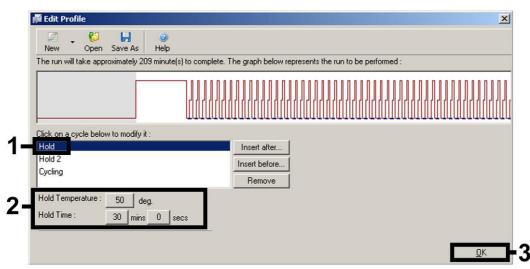


Figure 3. Editing the profile. Screenshot for the artus HI Virus-1 QS-RGQ Kit is shown as an example.

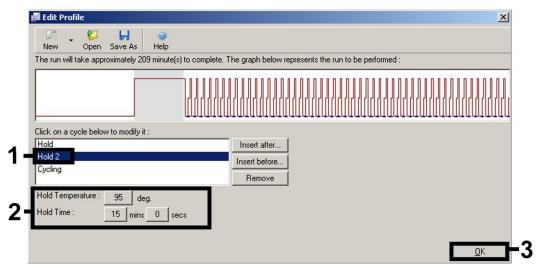
artus QS-RGQ Kit	BK Virus, CMV, EBV, HSV-1/2, VZV	НВV	HCV, HI Virus-1
Hold	Temperature: 95 deg	Temperature: 95 deg	Temperature: 50 deg
	Time: 10 mins	Time: 10 mins	Time: 30 mins
Hold 2	Step not required	Step not required	Temperature: 95 deg
			Time: 15 mins
Cycling	45 times	45 times	50 times
	95 deg for 15 secs	95 deg for 15 secs	95 deg for 30 secs
	65 deg for 30 secs	55 deg for 30 secs	50 deg for 60 secs
	72 deg for 20 secs	72 deg for 15 secs	72 deg for 30 secs
	Make sure to activate the touchdown function for 10 cycles in the annealing step.		

QIAsymphony RGQ Protocol Sheet: Settings to run artus QS-RGQ Kits (Rotor-Gene Q software 2.1, or higher)page 3 of 7

**Note**: See also the relevant QIAsymphony RGQ Application Sheet at <u>www.qiagen.com/products/qiasymphonyrgq.aspx</u>.

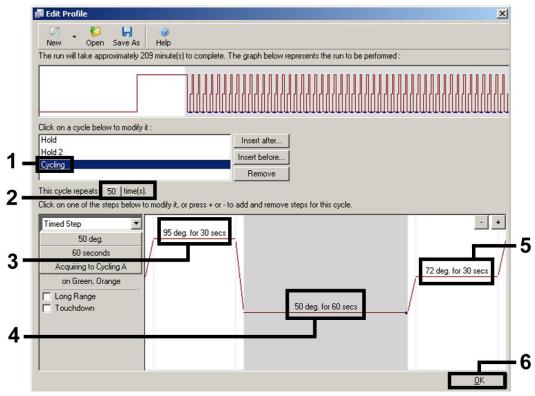


**Figure 4. Reverse transcription of the RNA.** Screenshot for the *artus* HI Virus-1 QS-RGQ Kit is shown as an example. **Note**: This step may differ for other *artus* QS-RGQ Kits. See Table 1 for the specific information for each *artus* QS-RGQ Kit.



**Figure 5. Initial activation of the hot-start enzyme.** Screenshot for the *artus* HI Virus-1 QS-RGQ Kit is shown as an example. **Note**: This step may differ for other *artus* QS-RGQ Kits. See Table 1 for the specific information for each *artus* QS-RGQ Kit.

QIAsymphony RGQ Protocol Sheet: Settings to run artus QS-RGQ Kits (Rotor-Gene Q software 2.1, or higher) page 4 of 7



**Figure 6. Amplification of the DNA.** Screenshot for the *artus* HI Virus-1 QS-RGQ Kit is shown as an example. **Note**: This step may differ for other *artus* QS-RGQ Kits. See Table 1 for the specific information for each *artus* QS-RGQ Kit.

6. The detection range of the fluorescence channels has to be determined according to the fluorescence intensities in the PCR tubes. Click "Gain Optimisation" in the "New Run Wizard" dialog box (see Figure 3) to open the "Auto-Gain Optimisation Setup" dialog box. Set the calibration temperature to match the annealing temperature of the amplification program, and adjust the fluorescence channel sensitivities (Table 2 and example screenshot in Figure 7).

artus QS-RGQ Kit Temperature	BK Virus, VZV 65 degrees	CMV, EBV 65 degrees	HB∨ 55 degrees	HCV, HI Virus-1 50 degrees	HSV-1/2 65 degrees
Channel Settings	Green Orange	Green Yellow	Green Yellow	Green Orange	Green Orange Yellow

Table 2. "Auto-Gain Optimisation" settings for artus QS-RGQ Kits

**Note**: See also the relevant QIAsymphony RGQ Application Sheet at <u>www.qiagen.com/products/qiasymphonyrgq.aspx</u>.

QIAsymphony RGQ Protocol Sheet: Settings to run artus QS-RGQ Kits (Rotor-Gene Q software 2.1, or higher)page 5 of 7

Optimisatic	Auto-Gain Opti different gain le acceptable. Th chemistry you a	evels until it finds the range of fluore are performing.	I the fluoresence one at which the scence you are I	fluorescenc	e levels are	
	Set temperatur	e to  50 🚊 d	egrees.			
Optim	ise All 🛛 Op	timise Acquiring				
Perforr	n Optimisation B	efore 1st Acquisi	tion			
Perform	n Optimisation Al	50 Degrees At I	Beginning Of Rur	n		
Channel S	ettings :					
Channel S	ettings :				<b>_</b>	Add
		Litte Desetine	[ May Danafara	Min Chin		<u>A</u> dd
Name	Tube Position	Min Reading	Max Reading	Min Gain	▼ Max Gain	<u><u> </u></u>
		Min Reading 5FI 5FI	Max Reading 10Fl 10Fl	Min Gain -10 -10	▼ Max Gain 10 10	
Name Green	Tube Position	5FI	10FI	-10	10	<u><u> </u></u>
Name Green	Tube Position	5FI	10FI	-10	10	 <u>E</u> dit 
Name Green	Tube Position	5FI	10FI	-10	10	 <u>E</u> dit 
Name Green	Tube Position	5FI	10FI	-10	10	 <u>E</u> dit 
Name Green	Tube Position	5FI	10FI	-10	10	 <u>E</u> dit 

**Figure 7. Adjusting the fluorescence channel sensitivity.** Screenshot for the *artus* HI Virus-1 QS-RGQ Kit is shown as an example. **Note**: This step may differ for other *artus* QS-RGQ Kits. See Table 2 for the specific information for each *artus* QS-RGQ Kit.

7. The gain values determined by the channel calibration are saved automatically and are listed in the last menu window of the programming procedure (Figure 8). Click "Start Run".

New Run Wizard	Summary :			×
60000000	Setting Green Gain Orange Gain Rotor Sample Layout Reaction Volume (in microiters)	Value 4 8 72-Well Rotor A1-A8, 81-88, 50		
900 (1) 1 000 1 000 1 000	Once you've confirmed that your in begin the run. Click Save Templat Skip Wizard Sack</td <td>un settings are corre e to save settings fo</td> <td>ct, click Start Run to I future runs.</td> <td>Save Template</td>	un settings are corre e to save settings fo	ct, click Start Run to I future runs.	Save Template

Figure 8. Starting the run. Screenshot for the artus HI Virus-1 QS-RGQ Kit is shown as an example.

QIAsymphony RGQ Protocol Sheet: Settings to run artus QS-RGQ Kits (Rotor-Gene Q software 2.1, or higher) page 6 of 7

- 8. After starting the run, import the information from the cycler file by clicking the ("Open") button, or edit the samples manually.
- For interpretation of results, see the instrument user manual and the relevant QIAsymphony RGQ Application Sheet at www.qiagen.com/products/giasymphonyrgq.aspx.

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