Settings to run the *artus*[®] CT/NG QS-RGQ Kit (Rotor-Gene[®] Q software 2.1)

Check availability of new electronic labeling revisions at <u>www.qiagen.com/products/artusctngqsrgqkitce.aspx</u> before test execution. The current revision status is indicated by the issue date (format: month/year).

General information

CE 0197

artus CT/NG QS-RGQ Kit

Version 1, **REF** 4569365

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 artus CT/NG QS-RGQ Kit Handbook and relevant Application Sheet at <u>www.qiagen.com/products/artusctngqsrgqkitce.aspx</u>.
- Make sure that at the positive/negative controls as well as at least one negative control (NTC, provided in the kit and pipetted by the AS module) are included per PCR run. Because the artus CT/NG QS-RGQ Kit is a qualitative assay, no quantitation standards are supplied.

Procedure

- 1. Place the PCR tubes in the 72-Well Rotor of the Rotor-Gene Q.
- 2. Place empty PCR tubes with lids in empty rotor positions.

This will guarantee an optimal temperature distribution in the Rotor-Gene Q.

- 3. Lock the rotor with the locking ring.
- 4. Transfer the cycler file from the QIAsymphony AS to the Rotor-Gene Q computer.



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5. Open the "New Run Wizard" dialog box (Figure 1). Check the "Locking Ring Attached" box and click "Next".

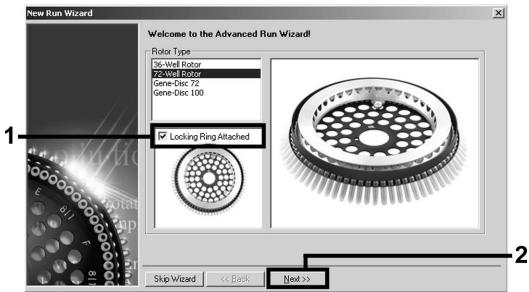


Figure 1. The "New Run Wizard" dialog box.

6. Select 25 for the PCR reaction volume and click "Next" (Figure 2).

Operator : Notes :	QIAGEN	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):	25	
Sample Layou		

Figure 2. Setting the general assay parameters.

 Click the "Edit Profile" button in the next "New Run Wizard" dialog box (Figure 3). Program the temperature profile as shown in Table 1, using the screenshots shown in Figures 3–5 as a guide.

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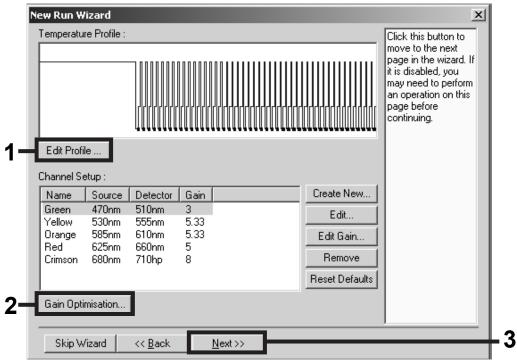


Figure 3. Editing the profile.

Table 1. Temperature profiles for the artus CT/NG QS-RGQ Kit

Hold	Temperature: 95 deg
	Time: 15 mins
Hold 2	Step not required
Cycling	45 times
	95 deg for 11 secs
	60 deg for 20 secs
	72 deg for 20 secs

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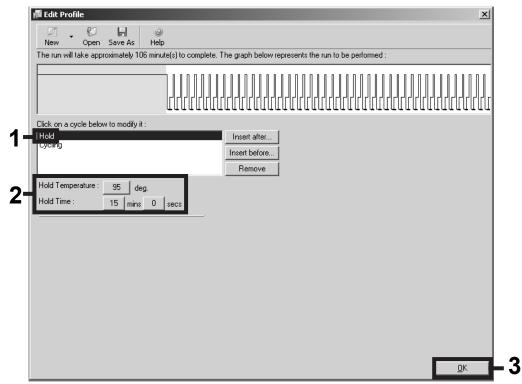


Figure 4. Initial activation of the hot-start enzyme.

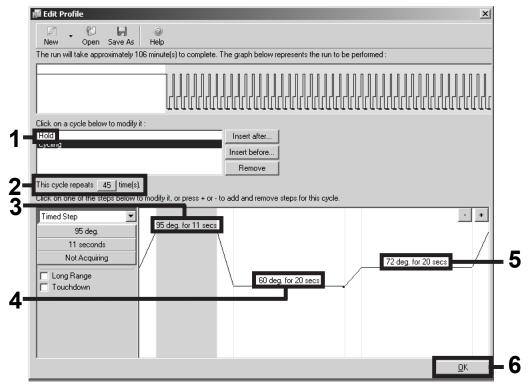


Figure 5. Amplification of the DNA.

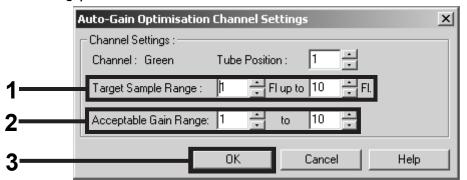
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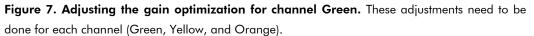
8. The detection range of the fluorescence channels has to be determined according to the fluorescence intensities in the PCR tubes. Click "Gain Optimisation" in the "New Run Wizard" dialog box (see Figure 3, page 3) to open the "Auto-Gain Optimisation Setup" dialog box. Set the calibration temperature to 60 to match the annealing temperature of the amplification program (Figure 6).

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Optimisatio	Auto-Gain O different gair acceptable.	n levels until it find	ad the fluoresence Is one at which the rescence you are	e fluorescend	e levels are i	
	Set tempera	ture to 60 🚊	degrees.			
Optim	ise All 🛛 🗍	Optimise Acquiring]			
Perform	n Optimisatior	Before 1st Acqui	sition			
_			t Beginning Of Ru	n		
Channel S	ettinas :					
	oningo.				_	Add
<u> </u>						
Name	Tube Positi	on 🛛 Min Reading	Max Reading	Min Gain	Max Gain	<u>E</u> dit
Green	1	1FI	10FI	1	10	Remove
Orange	1	1FI	10FI	1	10	<u><u> </u></u>
Yellow	1	1FI	10FI	1	10	Remove A
•						
			g			
<u>S</u> tart	Ma	nual	Close	<u>H</u> elp		

Figure 6. Adjusting the fluorescence channel sensitivity.

 Adjust the gain optimization. Choose a "Target Sample Range" from 1 Fl up to 10 Fl and an "Acceptable Gain Range" from 1 to 10 (Figure 7, example shown for channel Green). These adjustments need to be done for each channel (Green, Yellow, and Orange).





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10. Click the "Start" button to start gain optimization. After gain optimization is finished, click "Close" to return to the run wizard (Figure 8).

Optimisatio	Auto-Gain O different gair acceptable. chemistry yo	n Setup ptimisation will reac h levels until it finds The range of fluore u are performing. ture to 60 🚔 d	one at which the escence you are	e fluorescenc	e levels are	
	m Optimisation m Optimisation	Dptimise Acquiring Before 1st Acquisi At 60 Degrees At	ition	1		
	ettings :				•	Add
Name	ettings :	on Min Reading	Max Reading	Min Gain	▼ Max Gain	<u>A</u> dd <u>E</u> dit
Name Green	-	1FI	10FI	Min Gain	10	
Name Green Orange	-	1FI 1FI	10FI 10FI	Min Gain	10 10	 <u>E</u> dit
Name Green	-	1FI	10FI	Min Gain 1 1 1	10	<u> </u>
Name Green Orange	-	1FI 1FI	10FI 10FI	Min Gain 1 1 1	10 10	 <u>E</u> dit

Figure 8. Gain optimization.

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11. The gain values determined by the channel calibration are saved automatically and are listed in the last menu window of the programming procedure (Figure 9). Click "Start Run".

ew Run Wizard		×
Summary :		
Setting	Value	
Green Gain	3	
Orange Gain	5.33	
Yellow Gain	5.33	
Rotor	72-Well Rotor	
Sample Layout Reaction Volume (in microliters)	1, 2, 3, 25	
		Start Run
Dince you've confirmed that your ri begin the run. Click Save Templat		
Skip Wizard << <u>B</u> ack		

Figure 9. Starting the run.

- 12. After starting the run, import the information from the cycler file by clicking the ("Open") button, or edit the samples manually.
- 13. For interpretation of results, see the instrument user manual and the relevant **QIAsymphony RGQ Application Sheet at**

www.giagen.com/products/artusctnggsrggkitce.aspx.

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