QIAsymphony® RGQ Protocol Sheet

Settings to run *artus*® QS-RGQ Kits (Rotor-Gene® Q software 2.1, or higher)

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

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.

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.**

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

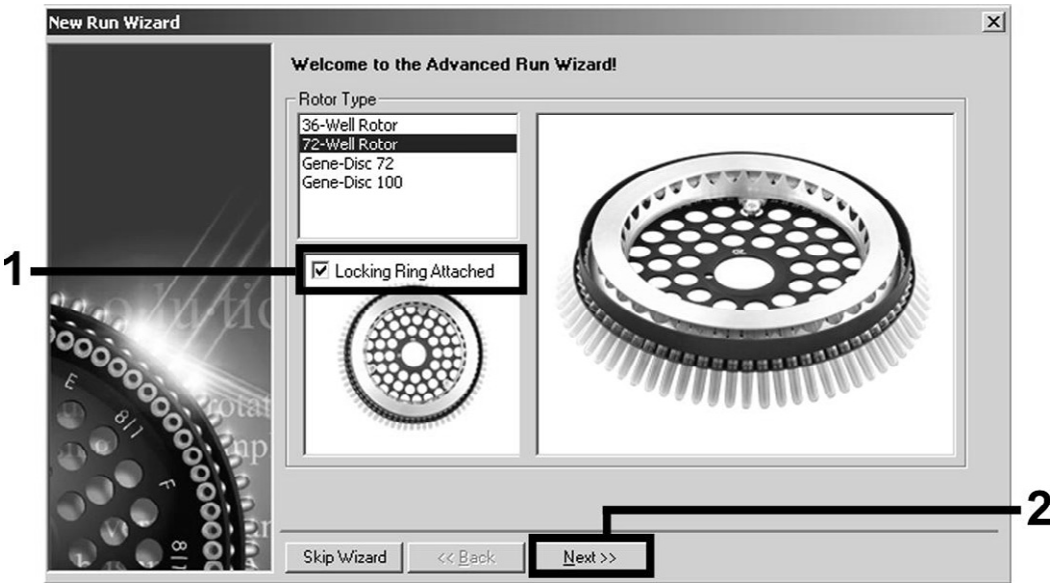

Figure 1. The "New Run Wizard" dialog box.

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

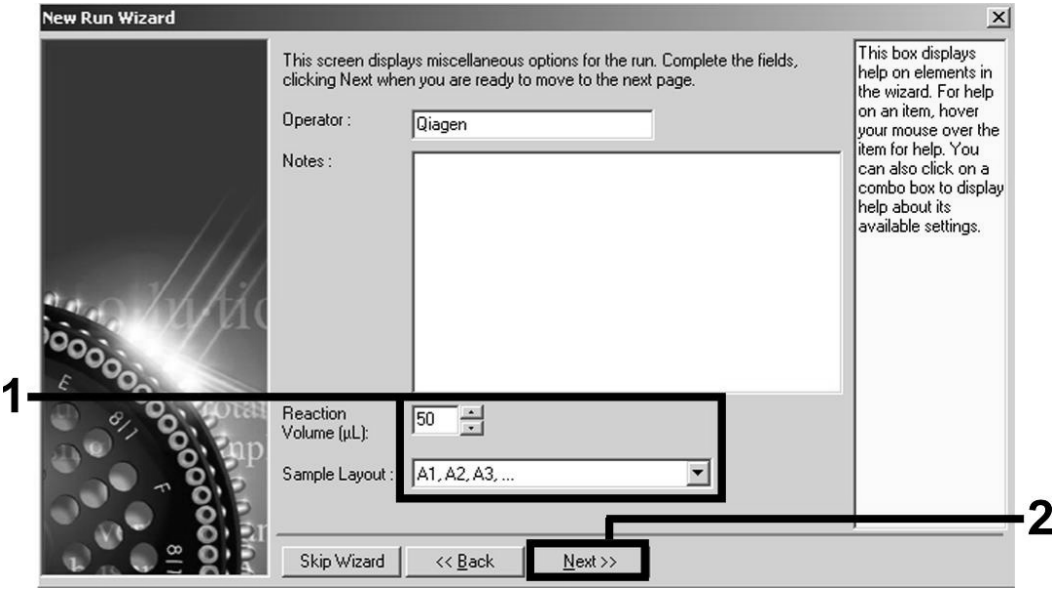

Figure 2. Setting the general assay parameters. Note: Even if the physical reaction volume is not 50 µl, make sure to select *50* for the reaction volume in the Rotor-Gene software.

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).**

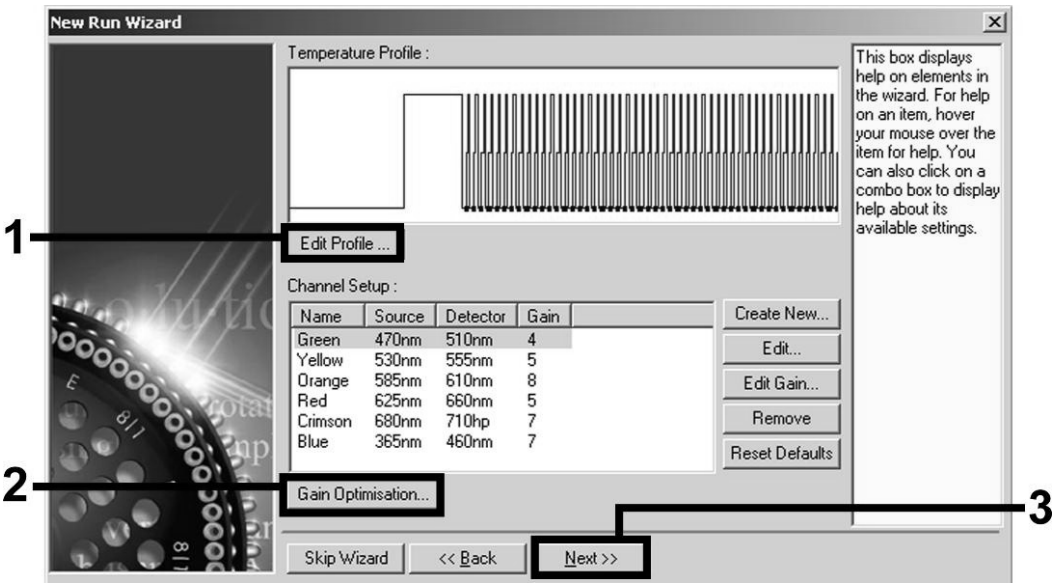

Figure 3. Editing the profile. Screenshot for the *artus* HI Virus-1 QS-RGQ Kit is shown as an example.

Table 1. Temperature profiles for *artus* QS-RGQ Kits

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

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

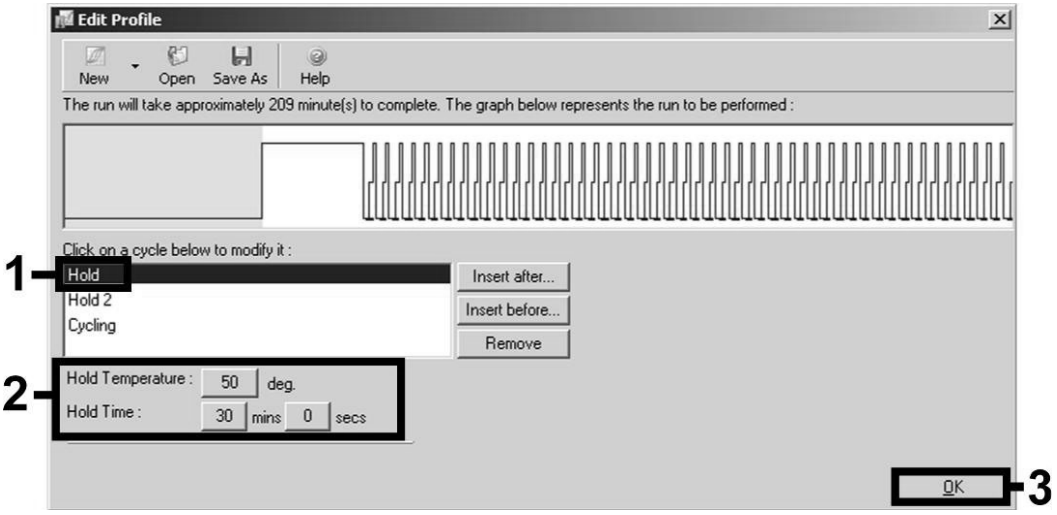

Figure 4. Reverse transcription of the RNA. Screenshot for the *artus* HI Virus-1 QS-RGQ Kit is shown as an example. **Note**: This step may differ for other *artus* QS-RGQ Kits. See Table 1 for the specific information for each *artus* QS-RGQ Kit.

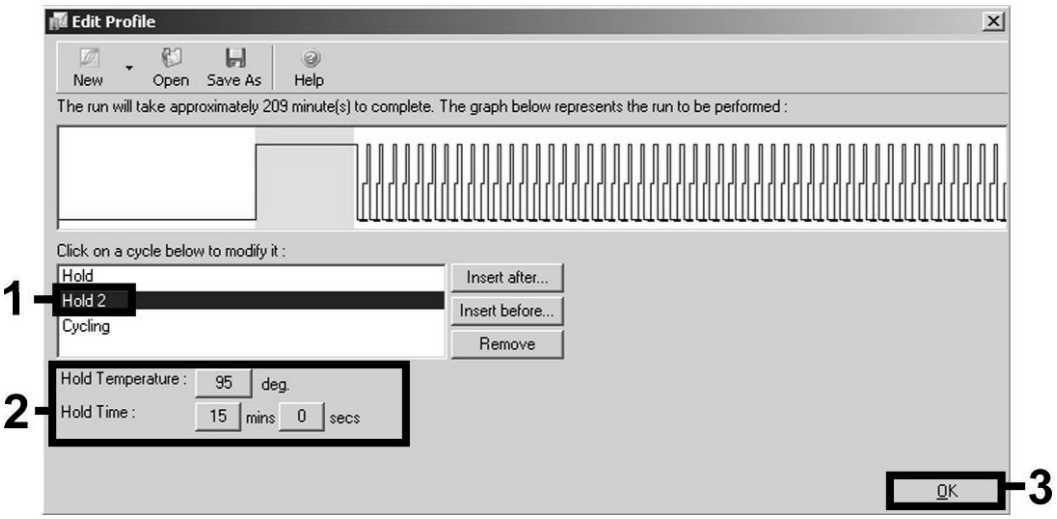

Figure 5. Initial activation of the hot-start enzyme. Screenshot for the *artus* HI Virus-1 QS-RGQ Kit is shown as an example. **Note**: This step may differ for other *artus* QS-RGQ Kits. See Table 1 for the specific information for each *artus* QS-RGQ Kit.

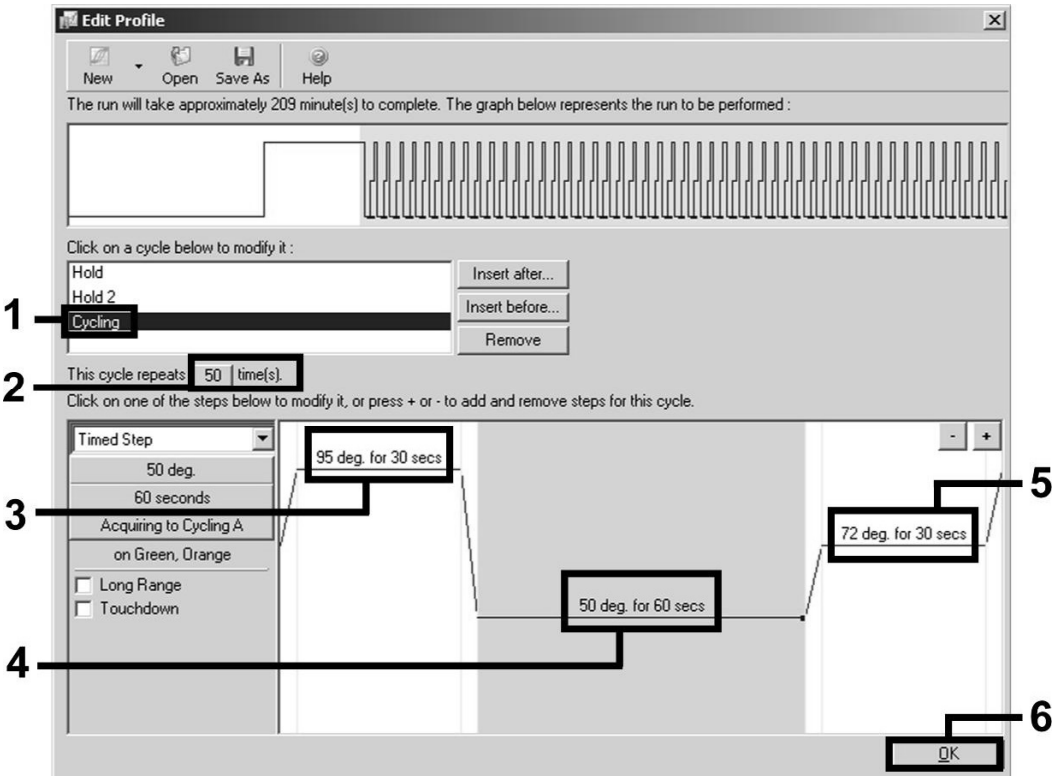

Figure 6. Amplification of the DNA. Screenshot for the *artus* HI Virus-1 QS-RGQ Kit is shown as an example. **Note**: This step may differ for other *artus* QS-RGQ Kits. See Table 1 for the specific information for each *artus* QS-RGQ Kit.

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).**

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

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

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

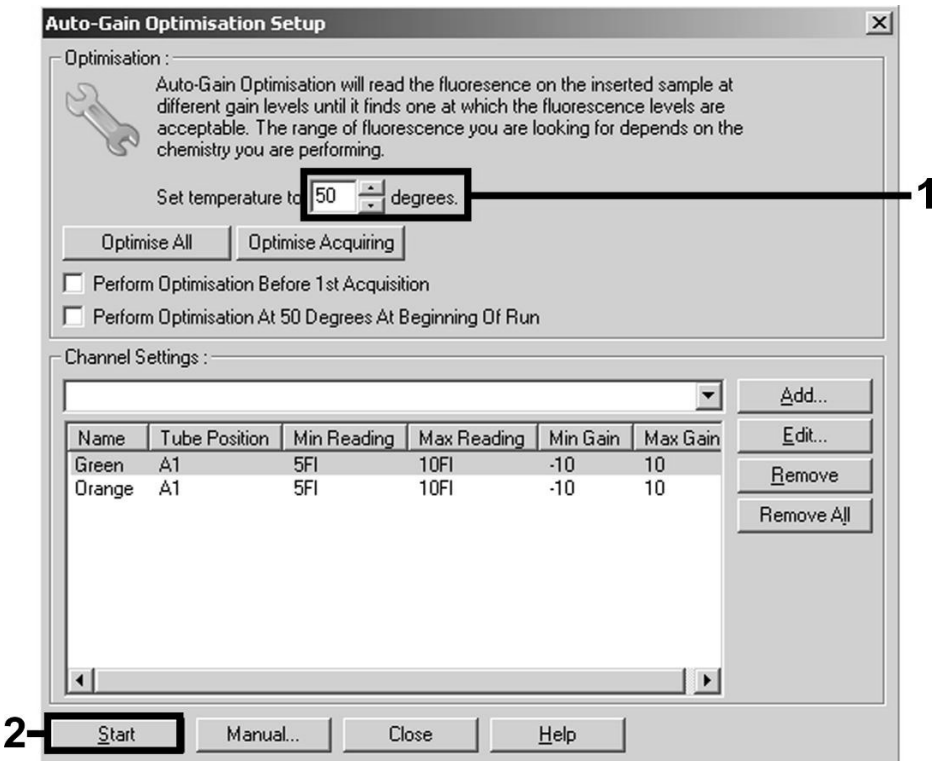

Figure 7. Adjusting the fluorescence channel sensitivity. Screenshot for the *artus* HI Virus-1 QS-RGQ Kit is shown as an example. **Note**: This step may differ for other *artus* QS-RGQ Kits. See Table 2 for the specific information for each *artus* QS-RGQ Kit.

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".**

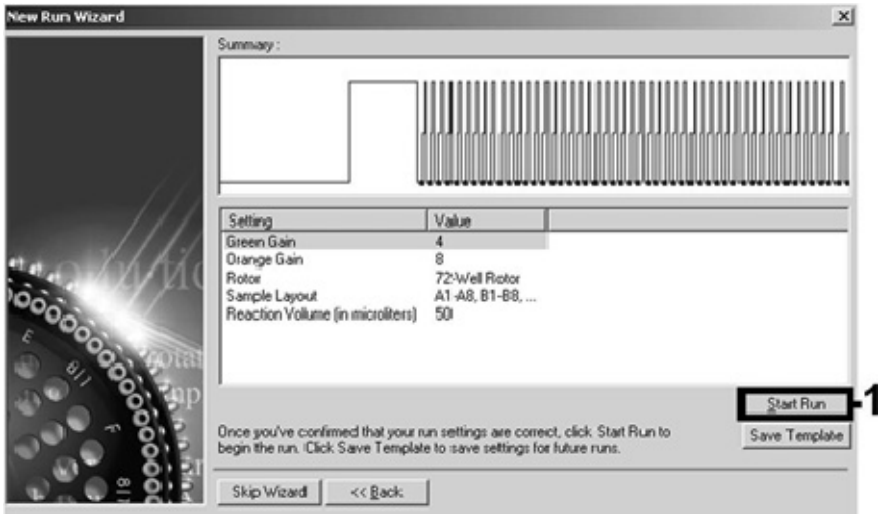

Figure 8. Starting the run. Screenshot for the *artus* HI Virus-1 QS-RGQ Kit is shown as an example.

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

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

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