

July 2020

Supplementary Protocol

Rotor-Gene® SYBR® Green PCR Demo Kit

Evaluation of the performance of QIAquant qPCR cyclers using the Rotor-Gene® SYBR® Green PCR Demo Kit

Intended use

The Rotor-Gene® SYBR® Green PCR Demo Kit (cat no. 204001) has been developed for use in demonstrating the high performance of the Rotor-Gene Q qPCR cycler. The kit is also suitable for the evaluation of the QIAquant instruments. This supplementary protocol explains how to use the kit to evaluate the reliability and reproducibility of gene quantification with the QIAquant qPCR cyclers.

For kit content, shipping and storage, product use limitations, safety information, quality control, and support, please refer to the applicable kit handbook available at www.qiagen.com and inserted in the kit.

Introduction to QIAquant

The QIAquant instruments enable quantitative real-time PCR in either intercalating dye or hydrolysis probe-based assays and in tubes, or in a 96- and 384-well plate format.

QIAquant instruments are all-round versatile systems for quantitative PCR applications such as expression analyses, genotyping, and the detection of pathogens. They combine high-quality thermal elements for fast heating and cooling ramping rates and a fast multi-channel detection system for shorter cycling times down to less than 30 minutes for 40 cycles.

The open system is compatible with chemistries from any supplier and plasticware in standard SBS formats, low-and normal-profile, and all skirting types.

For more information, visit the QIAquant product webpage at www.qiagen.com.

Principle of the procedure

SYBR® Green-based real-time PCR is carried out to quantify different copy numbers of a genomic DNA target. Each reaction consists of:

- Human genomic DNA template of a defined copy number
- SYBR® Green PCR Master Mix
- QuantiTect Primer Assay specific for the human G protein-coupled estrogen receptor 1 (GPER) gene

A standard curve is generated from the C_T values obtained from a set of standards (2000, 1000, 500, 250, and 125 copies; each standard is analyzed in quadruplicate). The standard curve is then used to determine the copy number for 2 unknown samples (500 and 250 copies; 4 to 24 replicates of each unknown sample are analyzed). In addition, 4 no-template control (NTC) reactions are carried out. Thus, a total of up to 72 reactions are run at the same time on the QIAquant.

Kit contents

Rotor-Gene SYBR® Green PCR Demo Kit	
Catalog no.	204001
2x Rotor-Gene SYBR® Green PCR Master Mix	1.7 ml
10x QuantiTect® Primer Assay For GPER	1 tube
Standards 1–5† (Genomic DNA)	1 tube each
Unknown samples 1 and 2‡ (Genomic DNA)	1 tube each
Buffer TE	1.9 ml
RNase-Free Water	1.9 ml

* Contains a mix of lyophilized forward and reverse primers. See Assay setup for instruction to reconstitute the assay TE and mix by vortexing the tube 4–6 times; if necessary, gently warm the tube to help the primers dissolve.

† Five standards providing 2000, 1000, 500, 250, or 125 copies of target DNA per reaction.

‡ Two unknown samples providing 500 or 250 copies of target DNA per reaction.

The Rotor-Gene SYBR® Green PCR Demo Kit includes enough reagents for 100 reactions of 20 µL or 200 reactions of 10 µL.

Materials required but not provided

- Strip tubes and caps or sample plate and foil in quantity sufficient for minimum 32 reactions.

For best performance, we recommend using qPCR compatible consumables with white wells and optically-clear cap or seal. Compatible 96-well plates (cat no. 209002), 384-well plates (cat no. 209001) and adhesive foils (cat no. 209003) can be purchased separately from QIAGEN.

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Procedures

Assay setup

Things to do before starting

- Thaw Buffer TE and mix by inverting the tube several times.
- Reconstitute the QuantiTect Primer Assay by briefly centrifuging the tube, adding 1.1 ml Buffer TE, and vortexing the tube 4–6 times. If necessary, gently warm the tube to help the primers dissolve.

Procedure

1. Thaw the 2x Rotor-Gene SYBR Green PCR Master Mix, standards, unknown samples, and RNase-free water. Mix well all solutions before use to avoid localized concentrations of salt.
2. Prepare a reaction mix according to Table 1.

Note: Thanks to the hot-start, it is not necessary to keep samples on ice during reaction setup or while programming the QIAquant cycler. In this experiment, thirty-two reactions will be run (quadruplicate reactions for 5 standards, 2 unknown samples, and one NTC). For an assay on the QIAquant 96 (2plex or 5plex), the reaction volume is set to 20 μ l and for the QIAquant 384 5plex, the reaction volume is set to 10 μ l. A reaction mix for thirty-six reactions should be prepared, as some reaction mix will be lost during pipetting.

Table 1. Reaction setup

	Volume per reaction		Volume to prepare for 32 reactions*		Final concentration
	10 μ l (384-well)	20 μ l (96-well)	360 μ l	720 μ l	
2x Master Mix	5 μ l	10 μ l	180 μ l	360 μ l	1x
10x QuantiTect Primer Assay	1 μ l	2 μ l	36 μ l	72 μ l	1x
RNase-Free Water	2 μ l	4 μ l	72 μ l	144 μ l	–
Template DNA (added at step 4)	2 μ l	4 μ l	72 μ l	144 μ l	Varies

* A reaction mix for 36 reactions should be prepared as some reaction mix will be lost during pipetting.

- Mix the reaction mix thoroughly, and dispense 8 μ l or 16 μ l into PCR tubes or plate.
- Add template DNA to the individual wells or PCR tubes containing the reaction mix according to Table 2.

Table 2. Recommended pipetting scheme

Reaction number	Template	Copy number
1–4	Standard 1	2000
5–8	Standard 2	1000
9–12	Standard 3	500
13–16	Standard 4	250
17–20	Standard 5	125
21–24	Unknown 1	500
25–28	Unknown 2	250
29–32	NTC†	–


† For NTC, add RNase-free water to the reaction mix instead of template DNA.

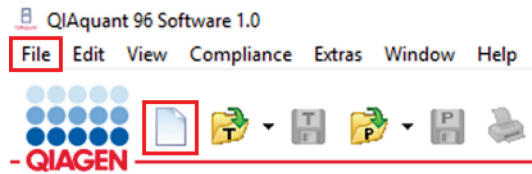
Manual pipetting steps can be avoided by using QIAgility®, a compact benchtop instrument that provides rapid, high-precision PCR setup. Mistakes in reaction setup due to human error are reduced and may be eliminated.

Setup run on the QIAquant Software

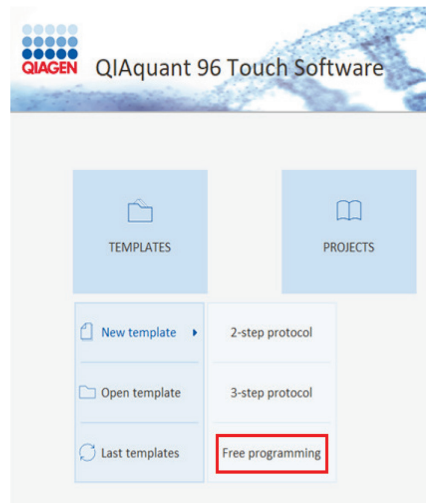
The operation of the QIAquant and the QIAquant Software should be done by users previously trained on the handling of the instrument and its software according to the laboratory safety standard and safety instruction listed in the user manual of the QIAquant instrument.

- Place tubes or plate in the QIAquant cyclers and close the lid.
- Open a new template.

- **QIAquant 96 software or QIAquant 384 software:** Open the QIAquant software. To open a new template, click **File > New** or click the  icon.



- **QIAquant 96 Touch software:** On the Home screen, tap **Templates > New Template > Free Programming**.



3. Program the QIAquant cyclers according to the program outlined in Table 3.

- **QIAquant 96 Software/QIAquant 384 Software:** Within the template file, go to the Thermal Cycler tab to enter the cycling profile (Figure 1). The channel for data collection can be set in the Scan tab (Figure 3).
Note: Optionally, a melting curve step can be added (Figure 2). The sample layout can be edited in the Samples tab, but can also be edited/changed after the run has finished (Figure 4).
- **QIAquant 96 Touch Software:** Go to the Thermal Cycler tab and enter the cycling profile (Figure 5). The channel for data collection can be selected in the Scan tab (Figure 7).
Note: Optionally, a melting curve step can be added (Figure 6). The sample layout can be edited in the Samples tab, but can also be edited/changed after the run has finished (Figure 8).

Table 3. Cycling conditions

Step	Time	Temperature	Notes
PCR initial activation	5 min	95°C	HotStar Taq Plus DNA Polymerase is activated by this heating step.
Two-step cycling:			Perform fluorescence data collection using the Blue channel with gain set to 5. Data acquisition should be performed during the annealing/extension step.
• Denaturation	5 s	95°C	
• Combined annealing/extension	QIAquant 96: 10 s QIAquant 384: 30 s	60°C	
• Number of cycles	35		
Optional: Melting curve analysis	15 s equilibration; Heating rate of 5°C/s	60–95°C, increments of 1°C	After PCR is completed, melting curve analysis can be performed to check the specificity of the reaction.

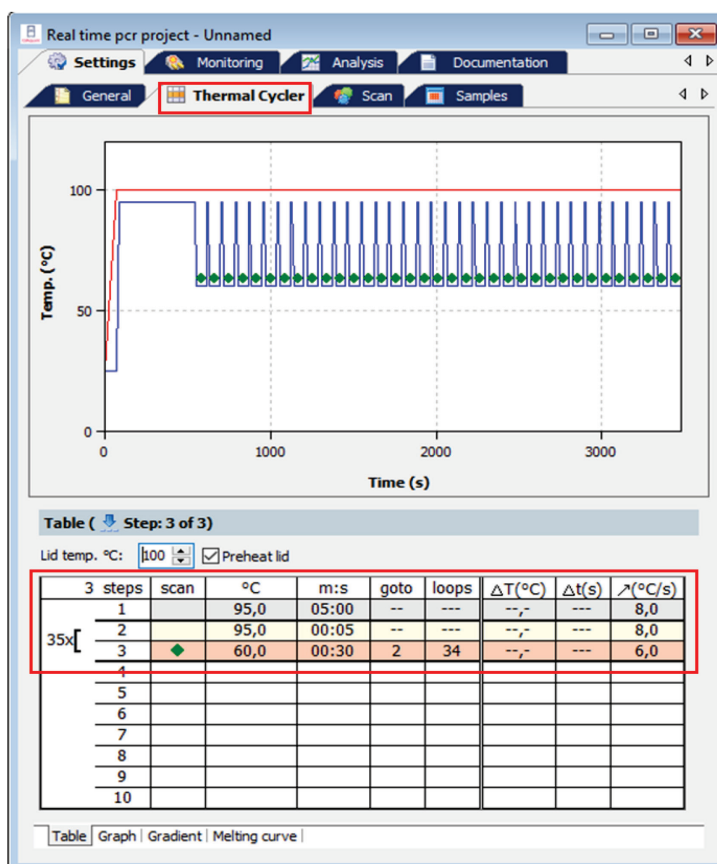


Figure 1. Setup of cycling profile with the QIAquant 96/QIAquant 384 Software. Under the Thermal Cycler tab, the cycling profile can be entered in the table view. To set the data acquisition, click within the scan column of the annealing/extension step (a green rectangle will appear).

Table (Step: 3 of 4)

Lid temp. °C: 100 Preheat lid

3 steps	scan	°C	m:s	goto	loops	ΔT(°C)	Δt(s)	λ(°C/s)
1		95,0	05:00	--	---	--,-	---	8,0
2		95,0	00:05	--	---	--,-	---	8,0
3	◆	60,0	00:30	2	34	--,-	---	6,0
4	◆	Melting curve 60 to 95 °C, 15 s with ΔT 1 °C						
5								
6								
7								
8								
9								
10								

Table | Graph | Gradient | **Melting curve**

Melting curve (Step: 3 of 4)

Start temp. (°C): Increment ΔT:

End temp. (°C): Heating rate (°C/s):

Equilibration (s):

active

Table | Graph | Gradient | **Melting curve**

Figure 2. Set up of melting curve step with the QIAquant 96/QIAquant 384 Software. After PCR is completed, melting curve analysis can be performed to check the specificity of the reaction. A melting curve step can be activated in the Melting curve tab.

Real time pcr project - Unnamed

Settings | Monitoring | Analysis | Documentation

General | Thermal Cycler | **Scan** | Samples

Pos.	Channel	Excitation	Detection	Dye	Gain	Measurement	Pass.	Ref.
1	Blue	465 nm	524 nm	FAM	5	◆		
2	Green	510 nm	565 nm	JOE	5			
4	Orange	560 nm	610 nm	ROX	5			
5	Red	625 nm	680 nm	Cy5	5			
6	NIR1	625 nm	710 nm	Cy5.5	5			

Meas. repeats: Color compensation:

Scan region according layout
 Define scan region manually

Figure 3. Selecting the channel for acquisition. For the fluorescence data collection, select **Blue** as the channel in the 'Scan' tab. A gain of 5 is set as default.

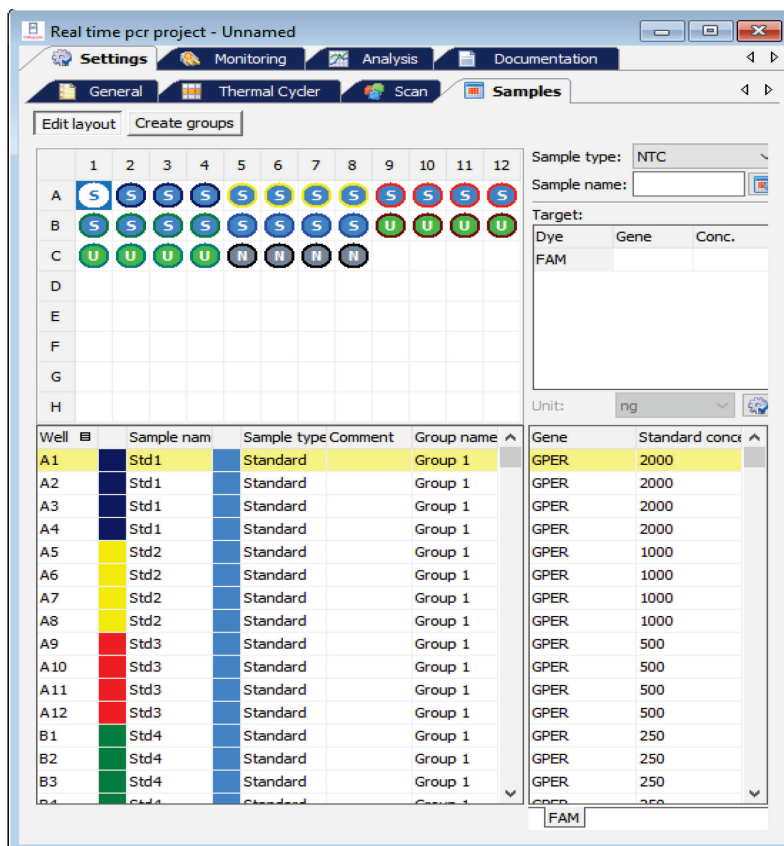


Figure 4. Sample layout setup on QIAquant 96/QIAquant 384 Software. The sample layout can be edited in the Samples tab. The layout can also be edited/changed after the run is finished.

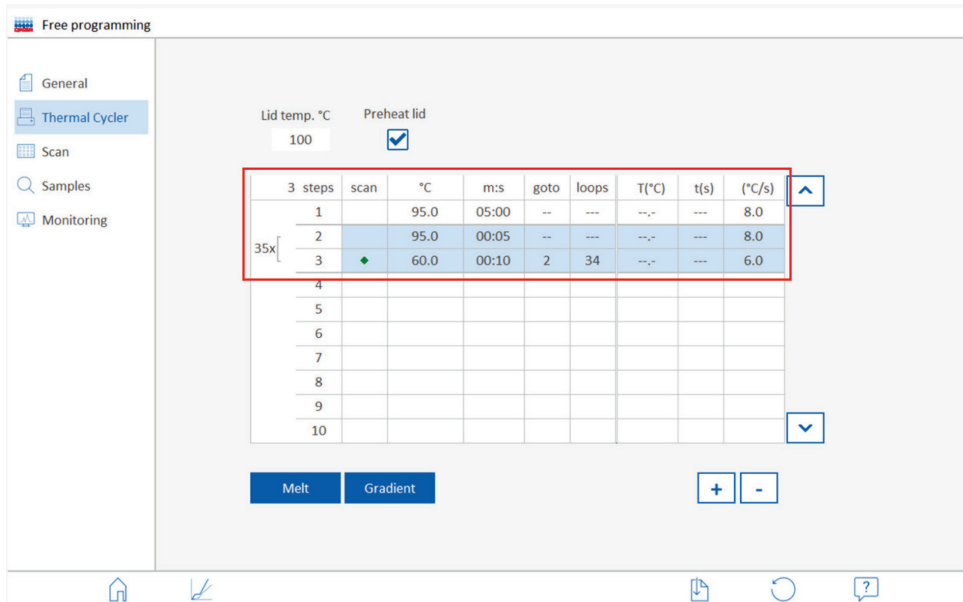


Figure 5. Setup of cycling profile using the QIAquant 96 Touch Software. On the Thermal Cycler tab of the Free Programming window, the cycling profile can be entered.

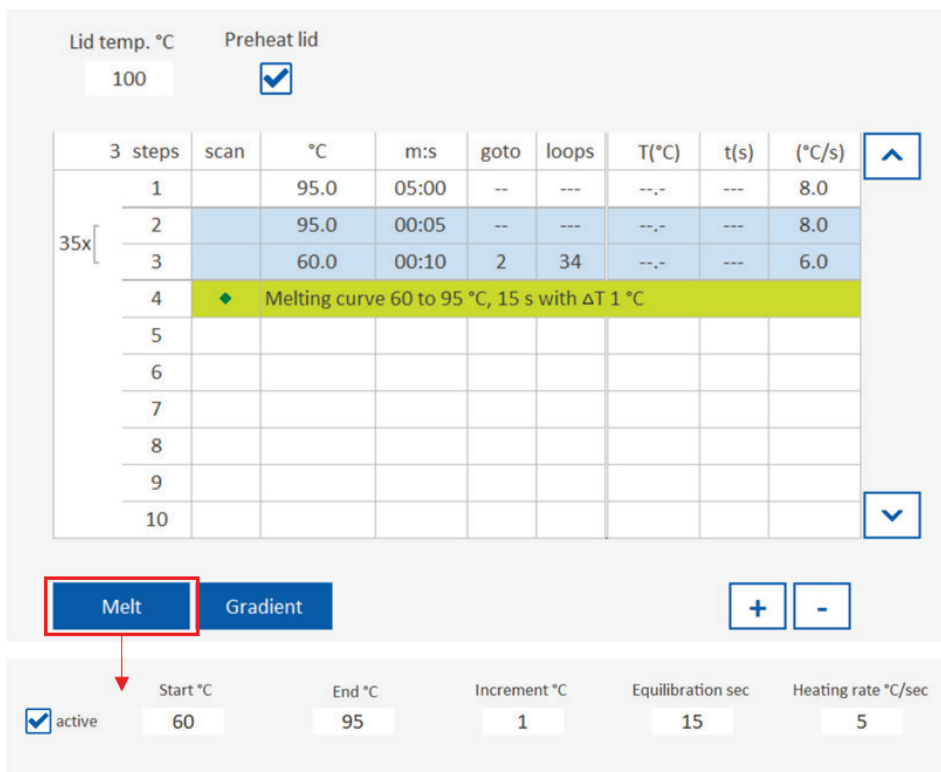


Figure 6. Setup of melting curve step with the QIAquant 96 Touch Software. After PCR is completed, melting curve analysis can be performed to check the specificity of the reaction. Click **Melt** to activate the melting curve step.

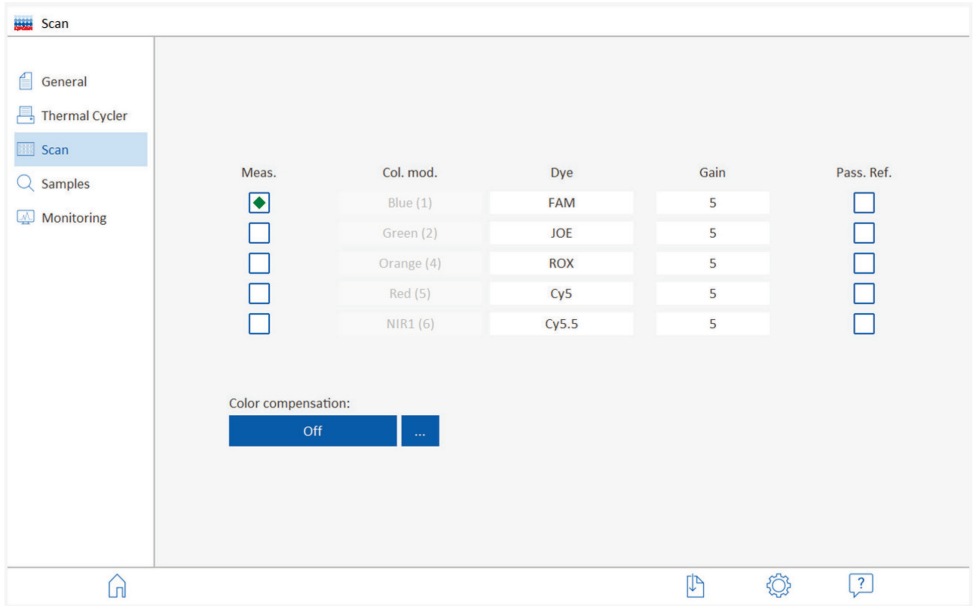


Figure 7. Selecting the channel for acquisition. Go to the Scan tab to select the **Blue** as the channel for the fluorescence data collection. A gain of **5** is set as default.

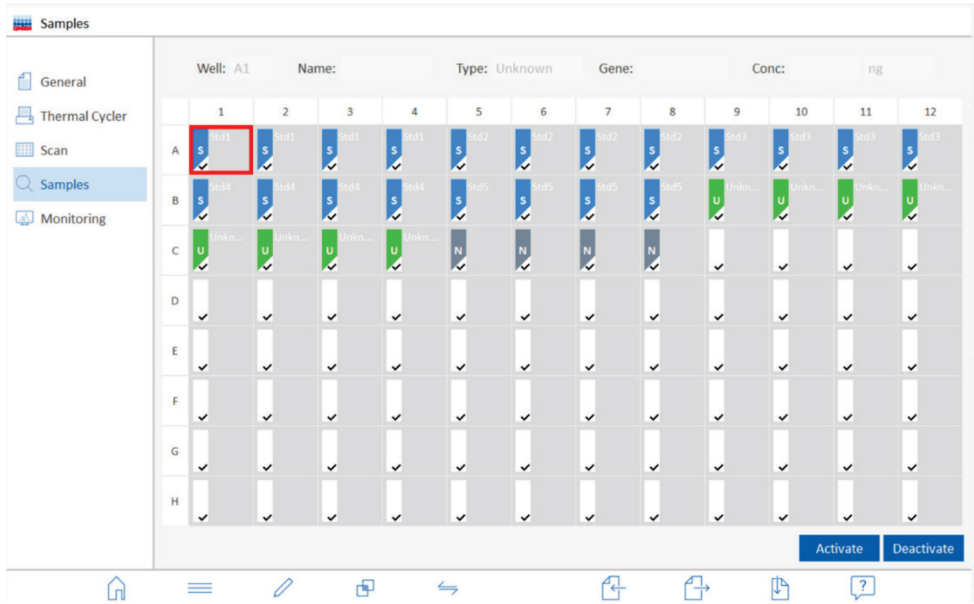




Figure 8. Sample layout setup on the QIAquant 96 Touch Software. The sample layout can be edited in the Samples tab. The layout can also be edited/changed after the run has finished.

4. Start the QIAquant cyclers.

- **QIAquant 96 Software/QIAquant 384 Software:** Click the  icon to start the measurement. The software will automatically switch to the Monitoring tab.
- **QIAquant 96 Touch Software:** Go to the Monitoring tab and click the  icon to start the measurement.

Document revision history

Date	Changes
July 2020	Initial release

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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