QuantiNova™ SYBR® Green PCR Kit

The QuantiNova SYBR Green PCR Kit (cat. nos. 208052, 208054, 208056) should be stored immediately upon receipt at –15 to –30°C in a constant-temperature freezer and protected from light. QuantiNova SYBR Green PCR Master Mix can also be stored protected from light at 2–8°C for up to 6 months, depending on the expiration date.

Further information

- *QuantiNova SYBR Green PCR Handbook*: www.qiagen.com/handbooks
- Safety Data Sheets: www.qiagen.com/safety
- Technical assistance: toll-free 00800-22-44-6000, or www.qiagen.com/contact

Notes before starting

- This protocol is optimized for quantification of gDNA or cDNA targets using SYBR Green I with any real-time cycler and condition for fluorescence normalization. ROX™ dye is required for various cyclers at the following concentrations:

  No requirement for ROX dye: Rotor-Gene®, Bio-Rad® CFX, Roche LightCycler® 480, and Agilent® Technologies Mx instruments

  Low concentration of ROX dye: Applied Biosystems® 7500, and ViiA®7 Real-Time PCR Systems

  High concentration of ROX dye: ABI PRISM® 7000, Applied Biosystems 7300, 7900, and StepOne™ Real-Time PCR Systems

- QN ROX Reference Dye is provided as a separate tube of passive reference dye for normalization of fluorescent signals on all real-time cyclers from Applied Biosystems. It should be diluted 1:10 in the 1x reaction when used on instruments requiring a high ROX concentration and 1:200 for instruments requiring a low ROX concentration.

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- The dye in QuantiNova Yellow Template Dilution Buffer allows tracking of pipetted samples in the qPCR. When added to the blue QuantiNova SYBR Green PCR Master Mix, the color changes from blue to green, indicating the successful inclusion of template. The use of this buffer is optional. It is provided as 100x concentrate and should be diluted (using water or Tris buffer) to obtain a final concentration of 1x within samples. To generate a template dilution series (e.g., for absolute quantification or determination of PCR efficiency), dilute the 100x concentrate (using water or Tris buffer) to obtain a final concentration of 1x QuantiNova Yellow Template Dilution Buffer. The buffer does not affect the sample stability and qPCR.
- For the highest efficiency in real-time PCR using SYBR Green I, amplicons should ideally be 60–200 bp in length.
- The two-step PCR cycling protocol, which has a denaturation step at 95°C and a combined annealing/extension step at 60°C, will also work for primers with a T_m well below 60°C.
- Always start with the cycling conditions and primer concentrations specified in this protocol.
- The PCR must start with an initial incubation step of 2 min at 95°C to activate the QuantiNova DNA Polymerase.

1. Thaw QuantiNova SYBR Green PCR Master Mix, QuantiNova Yellow Template Dilution Buffer, template gDNA or cDNA, primers, probes, QN ROX Reference Dye (if required), and RNase-free water. Mix the individual solutions.
2. Prepare a reaction mix according to Table 1. Due to the hot start, it is not necessary to keep samples on ice during reaction setup or while programming the real-time cycler.
3. Mix the reaction mix thoroughly, and dispense appropriate volumes into PCR tubes or the wells of a PCR plate.

Table 1. Reaction setup

Component	Volume/reaction: 96-well block, Rotor-Gene	Volume/reaction: 384-well block	Final concentration
Reaction mix			
2x SYBR Green PCR Master Mix	10 µl	5 µl	1x
QN ROX Reference Dye (Applied Biosystems instruments only)	2 µl/0.1 µl*	1 µl/0.05 µl*	1x
Primer A†	Variable	Variable	0.7 µM
Primer B†	Variable	Variable	0.7 µM
RNase-free water	Variable	Variable	–
Template gDNA or cDNA (added at step 4)	Variable	Variable	≤100 ng/reaction
Total reaction volume	**20 µl**	**10 µl**	**–**

* Corresponds to a 1:10 dilution for high ROX cyclers (i.e., ABI PRISM 7000, Applied Biosystems 7300, 7900, and StepOne Real-Time PCR Systems, and a 1:200 dilution for low-ROX dye cyclers (i.e., Applied Biosystems 7500 and ViiA7 Real-Time PCR Systems).

† If using QuantiTect® Primer Assays, the final concentration in the reaction should be 1x.

4. Add template gDNA or cDNA (≤100 ng/reaction) to the individual PCR tubes or wells containing the reaction mix.

   Note: For two-step RT-PCR, the volume of the cDNA added (from the undiluted reverse-transcription reaction) should not exceed 10% of the final PCR volume.

5. Program the real-time cycler according to Table 2.

   Note: Data acquisition should be performed during the combined annealing/extension step.

6. Place the PCR tubes or plates in the real-time cycler, and start the cycling program.

Table 2. Cycling conditions

Step	Time	Temperature	Ramp rate
PCR initial heat activation	2 min	95°C	Maximal/fast mode
2-step cycling:			
Denaturation	5 s	95°C	Maximal/fast mode
Combined annealing/extension	10 s*	60°C†	Maximal/fast mode
Number of cycles	35–40‡		
Melting curve analysis§			

* If your cycler does not accept this short time for data acquisition, use the shortest acceptable time.

† This temperature should also be used for QuantiTect Primer Assays and for all primer sets with a *T*m. well below 60°C.

‡ The number of cycles depends on the amount of template DNA.

§ Melting curve analysis is an analysis step built into the software of real-time cyclers. To perform the analysis, follow instructions provided by the supplier.

7. Perform a melting curve analysis of the PCR product(s).

   Note: We strongly recommend routinely performing this analysis, which is built into the software of real-time cyclers, to verify the specificity and identity of PCR products.

   Optional: Check the specificity of PCR product(s) by agarose gel electrophoresis.