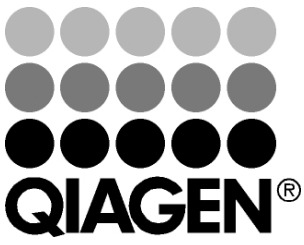
November 2013

QuantiNova™ SYBR® Green PCR Handbook

For highly sensitive, ultrafast, quantitative, real-time PCR and two-step RT-PCR using SYBR Green



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QIAGEN Sample and Assay Technologies

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Contents

Kit Contents

QuantiNova SYBR Green PCR Kit	**(100)**	**(500)**	**(2500)**
Catalog no.	**208052**	**208054**	**208056**
Number of reactions (20 µl/10 µl)	**100/ 200**	**500/ 1000**	**2500/ 5000**
2x QuantiNova SYBR Green PCR Master Mix, which contains: ■ QuantiNova DNA Polymerase (comprising *Taq* DNA Polymerase, QuantiNova Antibody, and QuantiNova Guard) ■ QuantiNova SYBR Green PCR Buffer ■ dNTP mix (dATP, dCTP, dGTP, dTTP)	1 ml	3 x 1.7 ml	15 x 1.7 ml
QuantiNova Yellow Template Dilution Buffer	500 µl	500 µl	5 x 500 µl
QN ROX™ Reference Dye	250 µl	1 ml	5 x 1 ml
RNase-Free Water	1.9 ml	1.9 ml	5 x 1.9 ml
Quick-Start Protocol	1	1	1

Storage

The QuantiNova SYBR Green PCR Kit is shipped on dry ice. Store immediately upon receipt at –15 to –30°C in a constant-temperature freezer and protect from light. When stored under these conditions and handled correctly, kit performance is guaranteed until the expiration date (see the quality-control label inside the kit box or on the kit envelope). QuantiNova SYBR Green PCR Master Mix, QuantiNova Yellow Template Dilution Buffer, and QN ROX Reference Dye should be stored protected from light and can be stored at 2–8°C for up to 6 months, depending on the expiry date.

If desired, QN ROX Reference Dye can be added to 2x QuantiNova SYBR Green PCR Master Mix for long-term storage. For details, see "Adding ROX dye to the master mix", page 11.

Intended Use

The QuantiNova SYBR Green PCR Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

24-hour emergency information

Chemical emergency or accident assistance is available 24 hours a day from:

CHEMTREC

USA & Canada ▪ Tel: 1-800-424-9300

Outside USA & Canada ▪ Tel: +1-703-527-3887 (collect calls accepted)

Product Specifications

2x QuantiNova SYBR Green PCR Master Mix contains:

Component	Description
QuantiNova DNA Polymerase	QuantiNova DNA Polymerase is a modified form of a recombinant 94 kDa DNA polymerase, originally isolated from *Thermus aquaticus*. QuantiNova DNA Polymerase is provided in an inactive state and has no enzymatic activity at ambient temperature. The enzyme is activated by a 2-minute, 95°C incubation step.
QuantiNova SYBR Green PCR Buffer	Contains Tris, NH_4^+, K^+, Mg^{2+}, and additives enabling fast cycling
dNTP mix	Contains dATP, dCTP, dGTP, and dTTP of ultrapure quality
Fluorescent dye	SYBR Green I
QN ROX Reference Dye	Optimized concentration of fluorescent dye for normalization of fluorescent signals on all instruments from Applied Biosystems®
QuantiNova Yellow Template Dilution Buffer	Ultrapure quality, PCR-grade
RNase-Free Water	Ultrapure quality, PCR-grade

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QuantiNova SYBR Green PCR Kit is tested against predetermined specifications to ensure consistent product quality.

The QuantiNova SYBR Green PCR Kit is subjected to the following quality control tests:

Component	Quality control tests
2x QuantiNova SYBR Green PCR Master Mix	(See quality-control label inside kit lid for lot-specific values) PCR sensitivity and reproducibility assay: Sensitivity, reproducibility, and specificity in real-time PCR are tested in a singleplex PCR assay in 20 µl reactions containing 10-fold dilutions of nucleic acid template.
QuantiNova DNA Polymerase (included in QuantiNova SYBR Green PCR Master Mix)	Efficiency, reproducibility, and stringency of hot-start in PCR are tested. Functional absence of exonucleases and endonucleases is tested.
Buffers and reagents (included in QuantiNova SYBR Green PCR Master Mix)	
QuantiNova SYBR Green PCR Buffer	Conductivity, density, pH, and ion concentrations are tested.
RNase-Free Water	Conductivity, pH, and RNase activities are tested.
QuantiNova Yellow Template Dilution Buffer	Dye concentration and RNase activities are tested.
QN ROX Reference Dye	Concentration is tested

Introduction

The QuantiNova SYBR Green PCR Kit provides highly sensitive and rapid real-time quantification of DNA and cDNA targets in an easy-to-use format. The kit can be used in real-time PCR of genomic DNA targets, and also in real-time, two-step RT-PCR of RNA targets following reverse transcription with, for example, the QuantiTect® Reverse Transcription Kit (see ordering information, page 25). The fluorescent dye, SYBR Green I, in the master mix enables analysis of many different targets without having to synthesize target-specific, labeled probes. High specificity and sensitivity in real-time PCR are achieved by the use of a novel hot-start enzyme, QuantiNova DNA Polymerase, together with a specialized real-time PCR buffer based on QIAGEN's proprietary PCR buffer technology. QuantiNova Guard, a novel additive, further improves the stringency of the antibody-mediated hot-start. The kit also includes a built-in control for visual identification of correct template addition. Additives in the PCR buffer ensure short cycling steps without loss of PCR sensitivity and efficiency.

The QuantiNova SYBR Green PCR Kit has been optimized for use with all currently available real-time cyclers. The QN ROX Reference Dye is provided in a separate tube and can be added if using a cycler that requires ROX as a passive reference dye.

Principle and Procedure

2x QuantiNova SYBR Green PCR Master Mix

The components of 2x QuantiNova SYBR Green PCR Master Mix include QuantiNova DNA Polymerase and QuantiNova SYBR Green PCR Buffer. The optimized master mix ensures ultrafast real-time PCR amplification with high specificity and sensitivity.

Novel, antibody-mediated hot-start mechanism

QuantiNova DNA Polymerase is provided in an inactive state and has no enzymatic activity at ambient temperature. This prevents the formation and extension of nonspecifically annealed primers during reaction setup and the first denaturation step, leading to high PCR specificity and accurate quantification. At low temperatures, the QuantiNova DNA Polymerase is kept in an inactive state by the QuantiNova Antibody and a novel additive, QuantiNova Guard, which stabilizes the complex. This improves the stringency of the hot-start.

Within 2 minutes of raising the temperature to 95°C, QuantiNova Antibody and QuantiNova Guard are denatured and QuantiNova DNA Polymerase is

activated, enabling PCR amplification (Figure 1). The hot-start allows reactions to be set up rapidly and conveniently at room temperature.

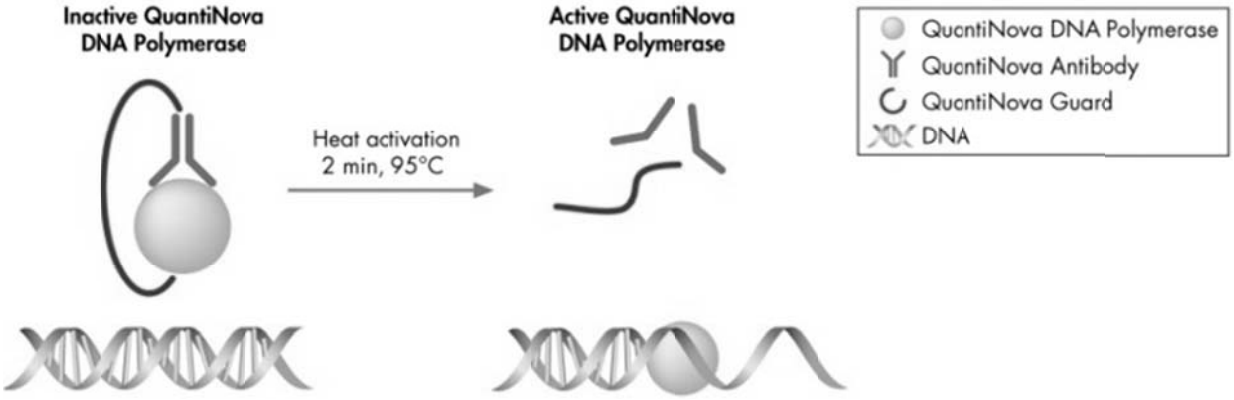

Figure 1. Principle of the novel QuantiNova hot-start mechanism. QuantiNova DNA Polymerase is kept in an inactive state by QuantiNova Antibody and QuantiNova Guard until the initial heat activation step.

Built-in visual control for correct pipetting

The master mix supplied with the QuantiNova SYBR Green PCR Kit contains an inert blue dye that does not interfere with the real-time PCR, but increases visibility in the tube or well. QuantiNova Yellow Template Dilution Buffer contains an inert yellow dye. When the template nucleic acid, diluted with the QuantiNova Yellow Template Dilution Buffer, is added to the master mix, the color of the solution changes from blue to green, providing a visual indication of correct pipetting. The use of the QuantiNova Yellow Template Dilution buffer is optional.

QuantiNova SYBR Green PCR Buffer

QuantiNova SYBR Green PCR Buffer is specifically designed for ultrafast, real-time PCR using SYBR Green I. The buffer additives allow short cycling times on any real-time cycler and a combined annealing/extension step of only 10 seconds. In addition, the unique composition of the buffer supports the melting behavior of DNA, enabling short denaturation and annealing/extension times.

QuantiNova SYBR Green PCR Buffer is also based on the unique QIAGEN PCR buffer system. The buffer contains a balanced combination of K^+ and NH_4^+, which promote a high ratio of specific to nonspecific primer binding during the annealing step of each PCR cycle. This creates stringent primer annealing conditions, leading to increased PCR specificity. When using this buffer, primer

annealing is only marginally influenced by the Mg^{2+} concentration, so optimization by titration of Mg^{2+} is not required.

SYBR Green I

The 2x QuantiNova SYBR Green PCR Master Mix contains an optimized concentration of the fluorescent dye SYBR Green I. SYBR Green binds all double-stranded DNA molecules, emitting a fluorescent signal on binding. The 2x QuantiNova SYBR Green PCR Master Mix can be stored at –20°C without loss of SYBR Green fluorescence activity. The excitation and emission maxima of SYBR Green I are at 494 nm and 521 nm, respectively, which are compatible with use on any real-time cycler.

Passive reference dye

For certain real-time cyclers, the presence of ROX passive reference dye in real-time PCR compensates for non-PCR–related variations in fluorescence detection. Fluorescence from ROX dye does not change during the course of real-time PCR, but provides a stable baseline to which PCR-related fluorescent signals are normalized. Thus, ROX dye compensates for differences in fluorescence detection between wells due to slight variations in reaction volume or to differences in well position. ROX dye does not interfere with real-time PCR as it is not involved in the reaction and has an emission spectrum that is different from the emission spectrum of SYBR Green I.

The use of ROX dye is necessary for instruments from Applied Biosystems. The QuantiNova SYBR Green PCR Kit is provided with a separate tube of QN ROX Reference Dye. It can be added to the real-time PCR if using a real-time cycler that uses ROX as a passive reference dye. The QN ROX Reference Dye should be diluted 1:10 in the 1x real-time PCR sample when used on instruments requiring a high ROX concentration and 1:200 for those instruments requiring a low ROX concentration. Refer to Table 1 for details on real-time cyclers that require low or high ROX concentrations. If required, QN ROX Reference Dye can be added to 2x QuantiNova SYBR Green PCR Master Mix for long-term storage (Table 2). For details, see “Adding ROX dye to the master mix”, page 11.

Table 1. Real-time cyclers requiring high/low concentrations of ROX

High ROX concentration (1:10 dilution of QN ROX Reference Dye in 1x reaction)	Low ROX concentration (1:200 dilution of QN ROX Reference Dye in 1x reaction)
ABI PRISM® 7000	Applied Biosystems 7500
Applied Biosystems 7300	Applied Biosystems ViiA™ 7
Applied Biosystems 7900	Applied Biosystems QuantStudio 12K Flex
Applied Biosystems StepOne™	
Applied Biosystems StepOne Plus	

Adding ROX dye to the master mix

If only Applied Biosystems cyclers will be used with the QuantiNova SYBR Green PCR Kit, QN ROX Reference Dye Solution can be added to the 2x QuantiNova SYBR Green PCR Master Mix for long-term storage, if desired. For information on the concentration of ROX required for Applied Biosystems instruments, refer to Table 1. For reaction setup with master mixes that already contain high concentration of added QN ROX Reference Dye, refer to "Appendix A: Reaction Setup Using Master Mix Containing a High Concentration of ROX", page 21.

Table 2. Addition of QN ROX Reference Dye to the master mix

Volume of 2x QuantiNova SYBR Green PCR Master Mix (w/o QN ROX Reference Dye)	Volume of QN ROX Reference Dye for high ROX concentration/low ROX concentration
1 ml	200/10 µl
1.7 ml	340/17 µl

cDNA synthesis for real-time, two-step RT-PCR

If quantifying cDNA targets with the QuantiNova SYBR Green PCR Kit, RNA must first be reverse-transcribed into cDNA. An aliquot of the reverse-transcription reaction is then transferred to another tube where real-time PCR takes place. This entire process is known as real-time, two-step RT-PCR, since reverse transcription and real-time PCR are carried out in separate tubes.

For reverse transcription, we recommend using the QuantiTect Reverse Transcription Kit. The kit provides a fast and convenient procedure, requiring only 20 minutes to synthesize first-strand cDNA and eliminate genomic DNA contamination. An optimized mix of oligo-dT and random primers enables cDNA synthesis from all regions of RNA transcripts, even from 5' regions of very long mRNA transcripts. cDNA yields are high, allowing sensitive detection of even low-abundance transcripts in real-time, two-step RT-PCR. For ordering information, see page 25.

Protocol: Real-Time PCR and Two-Step RT-PCR

Important points before starting

- Always start with the cycling conditions specified in this protocol, even if using previously established primer systems. The QuantiNova SYBR Green PCR Kit has been developed for use in a **two-step cycling** protocol, with a denaturation step at 95°C and a combined annealing/extension step at 60°C. This protocol will also work for primers with a T_m well below 60°C.
- For the highest efficiency in real-time PCR using SYBR Green, targets should ideally be 60–200 bp in length.
- The PCR must start with an initial incubation step of 2 minutes at 95°C to activate QuantiNova DNA Polymerase.
- For 96-well block cyclers, we recommend a final reaction volume of 20 µl. For 384-well block cyclers, we recommend a final reaction volume of 10 µl.
- 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.

Procedure

1. **Thaw the 2x QuantiNova SYBR Green PCR Master Mix, QuantiNova Yellow Template Dilution Buffer, template gDNA or cDNA, primers, QN ROX Reference Dye (if required), and RNase-Free water. Mix the individual solutions. Prepare a reaction mix according to Table 3.**

   Due to the hot-start, it is not necessary to keep samples on ice during reaction setup or while programming the real-time cycler.

Table 3. Reaction setup

Component	Volume/reaction		Final concentration
	96-well block	384-well block	
2x QuantiNova SYBR Green PCR Master Mix	10 µl	5 µl	1x
QN ROX Reference Dye (Applied Biosystems cycler 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 DNA 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.

2. **Mix the reaction mix thoroughly and dispense appropriate volumes into PCR vessels or plates.**
3. **Add template gDNA or cDNA (≤100 ng/reaction) to the individual PCR vessels or wells containing the reaction mix.**

   For two-step RT-PCR, the volume of the cDNA added (from the undiluted RT reaction) should not exceed 10% of the final PCR volume.
4. **Program your real-time cycler according to the program outlined in Table 4.**

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

Table 4. Real-time cycler conditions

Step	Time	Temperature	Ramp rate	Additional comments
PCR initial activation step	2 min	95°C	Maximal/ fast mode	QuantiNova DNA Polymerase is activated by this heating step
2-step cycling				
Denaturation	5 s	95°C	Maximal/ fast mode	
Combined annealing/ extension	10 s*	60°C	Maximal/ fast mode	Perform fluorescence data collection
Number of cycles	35–40‡			The number of cycles depends on the amount of template DNA
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.

5. **Place the PCR vessels or plates in the real-time cycler and start the cycling program.**
6. **Perform melting curve analysis of the PCR product(s).**

   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.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information or protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

	Comments and suggestions
No signal or one or more signals detected late in PCR	
a) Incorrect cycling conditions	Always start with the optimized cycling conditions specified in the protocols. Ensure that the cycling conditions include the initial step for activation of QuantiNova DNA Polymerase (95°C for 2 min), and the specified times for denaturation and annealing/extension.
b) QuantiNova DNA Polymerase not activated	Ensure that the cycling program includes the QuantiNova DNA Polymerase activation step (2 min at 95°C) as described in the protocol.
c) Pipetting error or missing reagent	Check the concentrations and storage conditions of the reagents, including primers, and template nucleic acid. See Appendix B (page 22) for details on evaluating the concentration of primers and. Repeat the PCR. Use the provided QuantiNova Yellow Template Dilution Buffer to prevent errors during reaction setup.
d) Wrong or no detection step	Ensure that fluorescence detection takes place during the combined annealing/extension step.
e) Primer concentration not optimal	Use each primer at an optimal concentration of 0.7 µM. If using a 10x QuantiTect Primer Assay, the final concentration in the reaction should be 1x. Check the concentrations of primers by spectrophotometry (see Appendix B, page 22).

	Comments and suggestions
f) Problems with starting template	Check the concentration, storage conditions, and quality of the starting. If necessary, make new serial dilutions of template nucleic acid from the stock solutions. Repeat the PCR using the new dilutions.
g) Insufficient amount of starting template	Increase the amount of template, if possible. Ensure that sufficient copies of the target nucleic acids are present in your sample.
h) Insufficient number of cycles	Increase the number of cycles.
i) Reaction volume too high	For 96-well block cyclers, we recommend a final reaction volume of 20 µl. For 384-well block cyclers, we recommend a final reaction volume of 10 µl.
j) PCR product too long	For optimal results, PCR products should be between 60 and 200 bp.
k) Primer design not optimal	Check for PCR products by melting curve analysis or gel electrophoresis. If no specific PCR products are detected, review the primer design guidelines (see Appendix B, page 22). Alternatively, use QuantiTect Primer Assays, which are predesigned primer sets for real-time RT-PCR (see ordering information, page 25).
l) No detection activated	Check that fluorescence detection was activated in the cycling program.
m) Primers degraded	Check for possible degradation of primers on a denaturing polyacrylamide gel.

	Comments and suggestions
n) **RT-PCR only:** Volumes of RT reaction added were too high	High volumes of RT reaction added to the PCR may reduce amplification efficiency. Generally, the volume of undiluted reverse-transcription reaction added should not exceed 10% of the final PCR volume. If you need to use a large volume of reverse-transcription reaction as template, determine the maximum acceptable volume for the assay being carried out.
Applied Biosystems, Bio-Rad®, QIAGEN, and Agilent® systems only:	
Wrong detection channel/filter chosen	Ensure that the correct detection channel is activated or the correct filter set is chosen for SYBR Green I.
Primer dimers and/or nonspecific PCR products	
a) Mg^{2+} concentration adjusted	Do not adjust the Mg^{2+} concentration in 2x QuantiNova SYBR Green PCR Master Mix.
b) Primer design not optimal	Check for PCR products by melting curve analysis or gel electrophoresis. If no specific PCR products are detected, review the primer design guidelines (see Appendix B, page 22). Alternatively, use QuantiTect Primer Assays, which are predesigned primer sets for real-time RT-PCR (see ordering information, page 25).
c) PCR product too long	For optimal results, PCR products should be between 60 and 200 bp. PCR products should not exceed 300 bp.
d) Primers degraded	Check for possible degradation of primers on a denaturing polyacrylamide gel.

	Comments and suggestions
e) Contamination of RNA sample with genomic DNA	Design primers that span exon-exon boundaries, so that only cDNA targets can be amplified and detected. Alternatively, use QuantiTect Primer Assays, which are predesigned primer sets that avoid amplification of genomic DNA where possible (see ordering information, page 25). Perform reverse transcription with the QuantiTect Reverse Transcription Kit, which provides cDNA synthesis with integrated genomic DNA removal. Alternatively, treat the RNA sample with DNase to digest the contaminating genomic DNA.
Increased fluorescence or C_T value for "No Template" control	
a) Contamination of reagents	Discard all the components of the assay (e.g., master mix, and primers). Repeat the assay using new components.
b) Contamination during reaction setup	Take appropriate precautions during reaction setup, such as using aerosol-barrier pipet tips.
High fluorescence in "No Reverse Transcription" control	
Contamination of RNA sample with genomic DNA	Design primers that span exon-exon boundaries, so that only cDNA targets can be amplified and detected. Perform reverse transcription with the QuantiTect Reverse Transcription Kit, which provides cDNA synthesis with integrated genomic DNA removal. Alternatively, treat the RNA sample with DNase to digest the contaminating genomic DNA.
Varying fluorescence intensity	
a) Contamination of real-time cycler	Decontaminate the real-time cycler according to the manufacturer's instructions.
b) Real-time cycler no longer calibrated	Recalibrate the real-time cycler according to the manufacturer's instructions.

	Comments and suggestions
All cycler systems:	
Wavy curve at high template amounts for highly expressed targets	In the analysis settings, reduce the number of cycles used for background calculation (if your real-time cycler allows you to do so) or reduce the amount of template.
Applied Biosystems instruments only:	
ΔRn values unexpectedly too high or too low	The concentration of the QN ROX Reference Dye is wrong. To choose the right ROX concentration for your cycler, refer to Table 1, page 11.

Appendix A: Reaction Setup Using Master Mix Containing a High Concentration of ROX

Note: This appendix is only relevant for a reaction setup using a master mix containing a high concentration of ROX. When using a master mix containing a low concentration of ROX, the volume of ROX added is negligible, follow the standard reaction setup as described in Table 3, page 14.

Table 5. Reaction setup

	Volume/reaction		
Component	**96-well block**	**384-well block**	**Final concentration**
2x QuantiNova SYBR Green PCR Master Mix (containing high concentration of ROX)	12 µl	6.0 µl	1x
Primer A	Variable	Variable	0.7 µM
Primer B	Variable	Variable	0.7 µM
Template DNA or cDNA	Variable	Variable	≤100 ng/ reaction
RNase-Free water	Variable	Variable	
Total reaction volume	**20 µl**	**10 µl**	

Appendix B: Assay Design and Handling Primers

Important factors for successful quantitative, real-time PCR include the design of optimal primer pairs, the use of appropriate primer concentrations, and the correct storage of primers.

Assay design

For guaranteed results in gene expression analysis experiments, we recommend using QuantiTect Primer Assays (see ordering information, page 25). If designing your own primers, please follow the guidelines for the optimal design of primers given below

Since fluorescence from SYBR Green I increases strongly upon binding of the dye to any double-stranded DNA, it is particularly important to minimize nonspecific primer annealing by careful primer design.

Primer sequence

- Length: 18–30 nucleotides.
- GC content: 40–60%.
- Always check the specificity of primers by performing a BLAST® search (www.ncbi.nlm.nih.gov/blast). Ensure that primer sequences are unique for your template sequence.
- Check that primers are not complementary to each other.
- Try to avoid highly repetitive sequences.
- Avoid complementarity of 2 or 3 bases at the 3' ends of primer pairs to minimize primer dimer formation.
- Avoid mismatches between the 3' end of primers and the template sequence.
- Avoid runs of 3 or more Gs and/or Cs at the 3' end. Avoid complementary sequences within a primer sequence and between the primer pair.

Product size

Ensure that the length of PCR products is 60–200 bp. Some longer amplicons may amplify efficiently with minimal optimization.

Handling and storing primers

Guidelines for handling and storing primers are given below. For optimal results, we recommend only combining primers of comparable quality.

Storage buffer

Lyophilized primers should be dissolved in a small volume of low-salt buffer to give a concentrated stock solution (e.g., 100 µM). We recommend using TE buffer (10 mM Tris·Cl, 1 mM EDTA, pH 8.0) for standard primers.

Storage

Primers should be stored in sterile, nuclease-free TE buffer in small aliquots at –20°C. Standard primers are stable under these conditions for at least 1 year. Repeated freeze–thaw cycles should be avoided, as they may lead to degradation.

Dissolving primers

Before opening a tube containing lyophilized primer, centrifuge the tube briefly to collect all material at the bottom of the tube. To dissolve the primer, add the required volume of sterile, nuclease-free TE buffer, mix, and leave for 20 minutes to allow the primer to completely dissolve. Mix again and determine the concentration by spectrophotometry as described below.

We do not recommend dissolving primers in water. They are less stable in water than in TE buffer and some may not dissolve easily in water.

Concentration

Spectrophotometric conversion for primers:

1 A_{260} unit = 20–30 µg/ml

To check primer concentration, the molar extinction coefficient (ε_{260}) can be used:

$A_{260} = \varepsilon_{260}$ x molar concentration of primer

If the ε_{260} value is not given on the data sheet supplied with the primers, it can be calculated from the primer sequence using the following formula:

$\varepsilon_{260} = 0.89 \times [(A \times 15{,}480) + (C \times 7340) + (G \times 11{,}760) + (T \times 8850)]$

Example

Concentration of diluted primer: 1 µM = 1 x 10^{-6} M

Primer length: 24 nucleotides with 6 each of A, C, G, and T bases

Calculation of expected A_{260}: 0.89 x [(6 x 15,480) + (6 x 7340) + (6 x 11,760) + (6 x 8850)] x (1 x 10^{-6}) = 0.232

The measured A_{260} should be within ±30% of the theoretical value. If the measured A_{260} is very different to the theoretical value, we recommend recalculating the concentration of the primers, or having the primers resynthesized.

Primer quality

The quality of 18–30mers can be checked on a 15% denaturing polyacrylamide gel;* a single band should be seen. Please contact QIAGEN Technical Services or your local distributor for a protocol.

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

Ordering Information

Product	Contents	Cat. no.
QuantiNova SYBR Green PCR Kit (100)	For 100 x 25 µl reactions: 1 ml 2x QuantiNova SYBR Green PCR Master Mix, 500 µl QuantiNova Yellow Template Dilution Buffer, 250 µl QN ROX Reference Dye, 1.9 ml RNase-Free Water	208052
QuantiNova SYBR Green PCR Kit (500)	For 500 x 25 µl reactions: 3 x 1.7 ml 2x QuantiNova SYBR Green PCR Master Mix, 500 µl QuantiNova Yellow Template Dilution Buffer, 1 ml QN ROX Reference Dye, 1.9 ml RNase-Free Water	208054
QuantiNova SYBR Green PCR Kit (2500)	For 2500 x 25 µl reactions: 15 x 1.7 ml 2x QuantiNova SYBR Green PCR Master Mix, 5 x 500 µl QuantiNova Yellow Template Dilution Buffer, 5 X 1 ml QN ROX Reference Dye, 5 x 1.9 ml RNase-Free Water	208056
Related products		
QuantiTect Primer Assays — for use in real-time RT-PCR with SYBR Green detection (search for and order assays at www.qiagen.com/GeneGlobe)		
QuantiTect Primer Assay (200)	For 200 x 50 µl reactions or 400 x 25 µl reactions: 10x QuantiTect Primer Assay (lyophilized)	Varies
QuantiTect Reverse Transcription Kit — for fast cDNA synthesis for sensitive real-time two-step RT-PCR		
QuantiTect Reverse Transcription Kit (50)	For 50 x 20 µl reactions: gDNA Wipeout Buffer, Quantiscript® Reverse Transcriptase, Quantiscript RT Buffer, RT Primer Mix, and RNase-Free Water	205311

Product	Contents	Cat. no.
QuantiTect Reverse Transcription Kit (200)	For 200 x 20 µl reactions: gDNA Wipeout Buffer, Quantiscript Reverse Transcriptase, Quantiscript RT Buffer, RT Primer Mix, and RNase-Free Water	205313
QuantiNova Probe PCR Kit — for highly sensitive, specific, and ultrafast, probe-based real-time PCR		
QuantiNova Probe PCR Kit (100)	For 100 x 25 µl reactions: 1 ml 2x QuantiNova Probe PCR Master Mix, 500 µl QuantiNova Yellow Template Dilution Buffer, 250 µl QN ROX Reference Dye, 1.9 ml RNase-Free Water	208252
QuantiNova Probe PCR Kit (500)	For 500 x 25 µl reactions: 3 x 1.7 ml 2x QuantiNova Probe PCR Master Mix, 500 µl QuantiNova Yellow Template Dilution Buffer, 1 ml QN ROX Reference Dye, 1.9 ml RNase-Free Water	208254
QuantiNova Probe PCR Kit (2500)	For 2500 x 25 µl reactions: 15 x 1.7 ml 2x QuantiNova Probe PCR Master Mix, 5 x 500 µl QuantiNova Yellow Template Dilution Buffer, 5 X 1 ml QN ROX Reference Dye, 5 x 1.9 ml RNase-Free Water	208256
DNeasy® Blood & Tissue Kit — for purification of total DNA from animal blood and tissues, and from cells, yeast, bacteria, or viruses		
DNeasy Blood & Tissue Kit (50)*	50 DNeasy Mini Spin Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	69504

Product	Contents	Cat. no.
RNeasy® Mini Kit — for purification of total RNA from animal cells, animal tissues, and yeast, and for RNA cleanup		
RNeasy Mini Kit (50)*	50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-Free Reagents and Buffers	74104
RNeasy Plus Mini Kit — for purification of total RNA from animal cells and tissues using gDNA Eliminator columns		
RNeasy Plus Mini Kit (50)	50 RNeasy Mini Spin Columns, 50 gDNA Eliminator Mini Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	74134
Oligotex® Direct mRNA Mini Kit — for purification of poly A+ mRNA directly from animal cells or tissues		
Oligotex Direct mRNA Mini Kit (12)*	For 12 mRNA minipreps: 420 µl Oligotex Suspension, Small Spin Columns, Collection Tubes (1.5 ml), RNase-Free Reagents and Buffers	72022
TurboCapture® 96 mRNA Kit — for rapid and easy mRNA purification from cultured cells in 96-well format		
TurboCapture 96 mRNA Kit (1)*	1 x TurboCapture 96 mRNA Plate, and RNase-Free Buffers	72250
AllPrep® DNA/RNA Mini Kit — for simultaneous purification of genomic DNA and total RNA from the same cell or tissue sample		
AllPrep DNA/RNA Mini Kit (50)	50 AllPrep DNA Mini Spin Columns, 50 RNeasy Mini Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	80204

* Other kit sizes and formats available; see www.qiagen.com.

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