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QuantiFast® SYBR® Green PCR Handbook

For fast, quantitative, real-time PCR and two-step RT-PCR using SYBR Green

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Kit Contents

QuantiFast SYBR Green PCR Kit	**(400)**	**(2000)**
Catalog no.	**204054**	**204056**
Number of reactions (25 µl/20 µl/10 µl)	**400/ 500/ 1000**	**2000/ 2500/ 5000**
2x QuantiFast SYBR Green PCR Master Mix, containing: ■ HotStarTaq® *Plus* DNA Polymerase ■ QuantiFast SYBR Green PCR Buffer ■ dNTP mix (dATP, dCTP, dGTP, dTTP) ■ ROX™ passive reference dye	3 x 1.7 ml	25 ml
RNase-Free Water	2 x 1.9 ml	20 ml
Handbook	1	1

Shipping and Storage

The QuantiFast SYBR Green PCR Kit is shipped on dry ice. The kit should be stored immediately upon receipt at –15°C to –30°C and protected from light. When the kit is stored under these conditions and handled correctly, performance is guaranteed until the expiration date (see the quality-control label inside the kit box or on the kit envelope). 2x QuantiFast SYBR Green PCR Master Mix can also be stored protected from light at 2–8°C for up to 1 month without showing any reduction in performance.

To maintain optimal performance of the QuantiFast SYBR Green PCR Kit for 2000 x 25 µl reactions, we recommend storing the 25 ml master mix as appropriately sized aliquots in sterile, polypropylene tubes.

Product Use Limitations

The QuantiFast 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.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the QuantiFast SYBR Green PCR Kit or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

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)

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each component of the QuantiFast SYBR Green PCR Kit is tested against predetermined specifications to ensure consistent product quality. See the quality-control label inside the kit box or on the kit envelope for lot-specific values.

Product Description

2x QuantiFast SYBR Green PCR Master Mix contains:

HotStarTaq *Plus* DNA Polymerase:	HotStarTaq *Plus* DNA Polymerase is a modified form of a recombinant 94 kDa DNA polymerase, originally isolated from *Thermus aquaticus*. HotStarTaq *Plus* DNA Polymerase is provided in an inactive state and has no enzymatic activity at ambient temperature. The enzyme is activated by a 5-minute, 95°C incubation step.
QuantiFast SYBR Green PCR Buffer:	Contains Tris·Cl, KCl, $(NH_4)_2SO_4$, $MgCl_2$, and additives enabling fast cycling, including Q-Bond®
dNTP mix:	Contains dATP, dCTP, dGTP, and dTTP of ultrapure quality
Fluorescent dyes:	SYBR Green and ROX
RNase-free water:	Ultrapure quality, PCR-grade

Introduction

The QuantiFast SYBR Green PCR Kit provides rapid real-time quantification of DNA and cDNA targets in an easy-to-handle 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 37). The fluorescent dye SYBR Green in the master mix enables the analysis of many different targets without having to synthesize target-specific labeled probes. High specificity and sensitivity in PCR are achieved by the use of the hot-start enzyme, HotStarTaq *Plus* DNA Polymerase, together with a specialized fast PCR buffer. The buffer also contains ROX dye, which allows fluorescence normalization on certain cyclers. Short cycling steps without loss of PCR sensitivity and efficiency are enabled by Q-Bond, a patent-pending additive in the PCR buffer.

The kit has been optimized for use with any real-time cycler, including cyclers with standard ramping rates and cyclers with rapid ramping rates:

- **Applied Biosystems**: ABI PRISM® 7000, 7700, and 7900, Applied Biosystems® 7300 and 7500, GeneAmp® 5700, ViiA™ 7 Real-Time PCR System, StepOne™ and StepOnePlus™ Real-Time PCR Systems
- **Bio-Rad**: iCycler iQ®, iQ5, MyiQ™, DNA Engine Opticon®, DNA Engine Opticon 2, CFX96™ Real-Time PCR Detection System, CFX384™ Real-Time PCR Detection System
- **Cepheid**: SmartCycler®
- **QIAGEN**: Rotor-Gene® cyclers
- **Eppendorf**: Mastercycler® ep *realplex*
- **Roche**: LightCycler® 1.x, LightCycler 2.0, LightCycler 480
- **Agilent (formerly Stratagene)**: Mx3000P®, Mx3005P®, Mx4000®

This handbook contains a general protocol for use with all these systems.

2x QuantiFast SYBR Green PCR Master Mix

The components of 2x QuantiFast SYBR Green PCR Master Mix include HotStarTaq *Plus* DNA Polymerase, QuantiFast SYBR Green PCR Buffer, SYBR Green, and ROX passive reference dye (see descriptions below).

HotStarTaq *Plus* DNA Polymerase

HotStarTaq *Plus* DNA Polymerase is a modified form of QIAGEN *Taq* DNA Polymerase, and is provided in an inactive state and has no enzymatic activity at ambient temperature. This prevents the formation of misprimed products and primer–dimers during reaction setup and the first denaturation step, leading to

high PCR specificity and accurate quantification. The enzyme is activated at the start of a reaction by a 5-minute, 95°C incubation step. The hot start enables reactions to be set up rapidly and conveniently at room temperature. In addition, the concentration of the polymerase in the master mix is optimized to allow short extension times in the combined annealing/extension step of each PCR cycle.

QuantiFast SYBR Green PCR Buffer

QuantiFast SYBR Green PCR Buffer is specifically designed for fast-cycling, real-time PCR using SYBR Green. A novel additive in the buffer, Q-Bond, allows short cycling times on standard cyclers and on fast cyclers with rapid ramping rates. Q-Bond increases the affinity of *Taq* DNA polymerases for short single-stranded DNA, reducing the time required for primer annealing to a few seconds. This allows a combined annealing/extension step of only 30 seconds. In addition, the unique composition of the buffer supports the melting behavior of DNA, enabling short denaturation and annealing/extension times.

QuantiFast SYBR Green PCR Buffer is also based on the unique QIAGEN PCR buffer system. The buffer contains a balanced combination of KCl and $(NH_4)_2SO_4$, which promotes 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 $MgCl_2$ concentration, so optimization by titration of Mg^{2+} is not required.

SYBR Green

2x QuantiFast SYBR Green PCR Master Mix contains an optimized concentration of the fluorescent dye SYBR Green. SYBR Green binds all double-stranded DNA molecules, emitting a fluorescent signal on binding. 2x QuantiFast SYBR Green PCR Master Mix can be stored at –15°C to –30°C without loss of SYBR Green fluorescence activity. The excitation and emission maxima of SYBR Green 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.

The use of ROX dye is necessary for all instruments from Applied Biosystems and is optional for the Mx3000P, Mx3005P, and Mx4000. Instruments from Bio-Rad, Cepheid, QIAGEN, Eppendorf, and Roche do not require ROX dye. The presence of ROX dye in the master mix does not interfere with real-time PCR on any instrument, since the dye is not involved in the reaction and has an emission spectrum completely different from that of SYBR Green.

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

If quantifying cDNA targets with the QuantiFast SYBR Green PCR Kit, RNA must first be reverse transcribed into cDNA. A portion 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 37.

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

Important points before starting

- The QuantiFast 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 5 minutes at 95°C** to activate HotStarTaq *Plus* DNA Polymerase.
- For 96-well block cyclers, we recommend a final reaction volume of 25 µl. For capillary cyclers, we recommend a final reaction volume of 20 µl. For 384-well block cyclers, we strongly recommend a final reaction volume of 10 µl.
- **Always start with the Mg^{2+} concentration as provided** in 2x QuantiFast SYBR Green PCR Master Mix.
- If using QuantiTect Primer Assays, the final concentration in the reaction should be 1x. Also, follow the cycling protocol in Table 2.
- If using the iCycler iQ, iQ5, or MyiQ, well factors must be collected at the beginning of each experiment. Well factors are used to compensate for any system or pipetting nonuniformity. For details, refer to the user manual supplied with the instrument or Appendix F (page 35).

Procedure

1. **Thaw 2x QuantiFast SYBR Green PCR Master Mix, template DNA or cDNA, primers, 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.

   Note: We strongly recommend starting with the Mg^{2+} concentration as provided in 2x QuantiFast SYBR Green PCR Master Mix.

Table 1. Reaction Setup

Component	Volume/reaction			Final concentration
	96-well block	Capillary cycler	384-well block	
2x QuantiFast SYBR Green PCR Master Mix	12.5 µl	10 µl	5 µl	1x
Primer A*	Variable	Variable	Variable	1 µM
Primer B*	Variable	Variable	Variable	1 µM
Template DNA or cDNA (added at step 4)	Variable	Variable	Variable	≤100 ng/ reaction
RNase-free water	Variable	Variable	Variable	
Total reaction volume	**25 µl**	**20 µl**	**10 µl**	

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

3. **Mix the reaction mix thoroughly and dispense appropriate volumes into PCR vessels or plates.**
4. **Add template DNA 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.
5. **Program your real-time cycler according to the program outlined in Table 2.**

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

Table 2. Real-Time Cycler Conditions

Step	Time	Temperature	Ramp rate	Additional comments
PCR initial activation step	5 min	95°C	Maximal/ fast mode	HotStarTaq *Plus* DNA Polymerase is activated by this heating step
Two-step cycling				
Denaturation	10 s	95°C	Maximal/ fast mode	
Combined annealing/ extension	30 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

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

6. **Place the PCR vessels or plates in the real-time cycler and start the cycling program.**
7. **Optional: Perform melting curve analysis of the PCR product(s) to verify their specificity and identity.**

   Melting curve analysis is an analysis step built into the software of real-time cyclers. Please follow instructions provided by the supplier.
8. **Optional: Check the specificity of the PCR product(s) by agarose gel electrophoresis.**

A step-by-step guide to software setup for your cycler can be found at www.qiagen.com/FastPCR.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocol in this handbook or molecular biology applications (see back cover for contact information).

	Comments and suggestions
No product, or product detected late in PCR, or only primer–dimers detected	
a) PCR annealing/ extension time too short	Use the recommended annealing/extension time of 30 s.
b) Mg^{2+} concentration adjusted	Do not adjust the Mg^{2+} concentration in 2x QuantiFast SYBR Green PCR Master Mix.
c) HotStarTaq *Plus* DNA Polymerase not activated	Ensure that the cycling program includes the HotStarTaq *Plus* DNA Polymerase activation step (5 min at 95°C) as described in the protocol.
d) Pipetting error or missing reagent	Check the concentrations and storage conditions of the reagents, including primers and template nucleic acid. See Appendix B, page 20, for details on evaluating the concentration of primers. Repeat the PCR.
e) Wrong or no detection step	Ensure that fluorescence detection takes place during the combined annealing/extension step.
f) Primer concentration not optimal	Use each primer at a concentration of 1 µM, as described in the protocol. 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 20).
g) Reaction volume too high	For 96-well block cyclers, we recommend a final reaction volume of 25 µl. For capillary cyclers, we recommend a final reaction volume of 20 µl. For 384-well block cyclers, we strongly recommend a final reaction volume of 10 µl.

	Comments and suggestions
h) Problems with starting template	Check the concentration, storage conditions, and quality of the starting template (see Appendix A, page 17). If necessary, make new serial dilutions of template nucleic acid from the stock solutions. Repeat the PCR using the new dilutions.
i) 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.
j) Insufficient number of cycles	Increase the number of cycles in steps of 5 cycles.
k) PCR product too long	For optimal results, PCR products should be between 60 and 200 bp. PCR products should not exceed 300 bp.
l) Primer design not optimal	Check for PCR products by melting curve analysis (see Appendix E, page 29) or gel electrophoresis. If no specific PCR products are detected, review the primer design guidelines (see Appendix B, page 20). Alternatively, use QuantiTect Primer Assays, which are predesigned primer sets for real-time RT-PCR (see Ordering Information, page 37).
m) No detection activated	Check that fluorescence detection was activated in the cycling program.
n) Primers degraded	Check for possible degradation of primers on a denaturing polyacrylamide gel.
o) ***RT-PCR only:*** Volumes of RT reaction added were too high	High volumes of RT reaction added to the PCR may reduce amplification efficiency and the linearity of the reaction. Generally, the volume of undiluted RT reaction added should not exceed 10% of the final PCR volume.
Applied Biosystems, Bio-Rad, QIAGEN, and Agilent systems only:	
p) Wrong detection channel/filter chosen	Ensure that the correct detection channel is activated or the correct filter set is chosen for SYBR Green.

	Comments and suggestions
LightCycler systems only:	
q) Chosen fluorescence gains too low	When using software versions earlier than 3.5, ensure fluorescence gain for channel 1 is set to "15".
Primer–dimers and/or nonspecific PCR products	
a) Mg^{2+} concentration adjusted	Do not adjust the Mg^{2+} concentration in 2x QuantiFast SYBR Green PCR Master Mix.
b) Primer design not optimal	Check for PCR products by melting curve analysis (see Appendix E, page 29) or gel electrophoresis. If no specific PCR products are detected, review the primer design guidelines (see Appendix B, page 20). Alternatively, use QuantiTect Primer Assays, which are predesigned primer sets for real-time RT-PCR (see Ordering Information, page 37).
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.
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 37). 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.
Applied Biosystems, Bio-Rad, and Agilent systems:	
f) 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.

	Comments and suggestions
LightCycler systems only:	
g) PCR mix not in capillary tip	Centrifuge the capillary to bring the PCR mix into the capillary tip.
h) Capillary not pushed down completely	Ensure that the capillary is completely pushed down in the LightCycler carousel.
i) Wrong detection channel	Make sure that the correct channel is chosen.

Appendix A: Preparation, Quantification, Determination of Quality, and Storage of DNA and RNA

Template preparation and quality

Since PCR consists of multiple rounds of enzymatic reactions, it is more sensitive to impurities such as proteins, phenol/chloroform, salts, and EDTA than single-step enzyme-catalyzed reactions. Purity of nucleic acid templates is particularly important for real-time PCR, since contaminants can interfere with fluorescence detection. QIAGEN offers a complete range of nucleic acid purification systems, ensuring the highest-quality templates for PCR, including QIAprep® Kits for rapid plasmid purification, QIAamp® and DNeasy® Kits for rapid purification of genomic DNA and viral nucleic acids, RNeasy® Kits for preparation of RNA from various sources, and Oligotex® Kits (low-throughput) and TurboCapture Kits (high-throughput) for mRNA purification. QIAGEN also offers a range of BioRobot® systems for automated purification of nucleic acids. For more information about these products, visit www.qiagen.com.

Determining concentration and purity of nucleic acids

The concentration of DNA and RNA should be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer. For accuracy, absorbance readings at 260 nm should fall between 0.15 and 1.0. Brief guides to spectrophotometric and molar conversion values for different nucleic acid templates are listed in Tables 3 and 4.

Table 3. Spectrophotometric Conversions for Nucleic Acid Templates

1 A_{260} unit*	Concentration (µg/ml)
Double-stranded DNA	50
Single-stranded DNA	33
Single-stranded RNA	40

* Absorbance at 260 nm = 1; 1 cm detection path.

Table 4. Molar Conversions for Nucleic Acid Templates

Nucleic acid	Size	pmol/µg	Molecules/µg
1 kb DNA	1000 bp	1.52	9.1×10^{11}
pUC19 DNA	2686 bp	0.57	3.4×10^{11}
pTZ18R DNA	2870 bp	0.54	3.2×10^{11}
pBluescript II DNA	2961 bp	0.52	3.1×10^{11}
Lambda DNA	48,502 bp	0.03	1.8×10^{10}
Typical mRNA	1930 nt	1.67	1.0×10^{12}
Genomic DNA			
Escherichia coli	4.7×10^{6}	3.0×10^{-4}	1.8×10^{8}†
Drosophila melanogaster	1.4×10^{8}*	1.1×10^{-5}	6.6×10^{5}†
Mus musculus (mouse)	2.7×10^{9}*	5.7×10^{-7}	3.4×10^{5}†
Homo sapiens (human)	3.3×10^{9}*	4.7×10^{-7}	2.8×10^{5}†

* Base pairs in haploid genome.

† For single-copy genes.

Note that absorbance measurements cannot discriminate between DNA and RNA. Depending on the method used for template preparation, DNA may be contaminated with RNA, or RNA may be contaminated with DNA, and either of these will result in misleadingly high A_{260} values. It is particularly important to bear this in mind when preparing standards for absolute quantification (see Appendix C, page 24).

The ratio between the absorbance values at 260 nm and 280 nm gives an estimate of the purity of DNA or RNA. To determine nucleic acid purity, we recommend measuring absorbance in 10 mM Tris·Cl,‡ pH 7.5. Pure DNA and RNA have A_{260}/A_{280} ratios of 1.8–2.0 and 1.9–2.1§ respectively. Lower ratios indicate the presence of contaminants such as proteins.

‡ 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), available from the product supplier.

§ Values up to 2.3 are routinely obtained for pure RNA (in 10 mM Tris·Cl, pH 7.5) with some spectrophotometers.

Storage of DNA and RNA

Purified RNA should be stored at –20°C or –70°C in RNase-free water. When RNA is purified using QIAGEN kits, no degradation is detectable for at least 1 year under these conditions. Purified DNA should be stored at –20°C or –70°C under slightly basic conditions (e.g., Tris·Cl, pH 8.0) because acidic conditions can cause hydrolysis of DNA. Diluted solutions of nucleic acids (e.g., dilution series used as standards) should be stored in aliquots and thawed once only. We recommend storage of aliquots in siliconized tubes where possible. This avoids adsorption of nucleic acids to the tube walls, which would reduce the concentration of nucleic acids in solution.

RT-PCR

When using the QuantiFast SYBR Green PCR Kit to perform two-step RT-PCR, the RNA must first be reverse transcribed into cDNA in an RT reaction. Failure of the subsequent PCR is often a result of the limitations of the RT reaction. On average, only 10–30% of template RNA molecules are reverse transcribed into cDNA. The expression level of the target RNA molecules and the relatively low efficiency of the RT reaction must be considered when calculating the appropriate amount of starting template for subsequent PCR. However, adding high volumes of the RT reaction to the PCR can affect C_T values. Generally RT volumes up to 10% of the total PCR volume do not affect results.

Total RNA or messenger RNA (mRNA) can be used as templates in RT reactions. We recommend RNeasy Kits for efficient purification of total RNA, Oligotex Kits (low-throughput) or TurboCapture Kits (high-throughput) for efficient purification of mRNA, and the QuantiTect Reverse Transcription Kit for reverse transcription (see page 37 for ordering information).

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 37). If designing your own primers, please follow the guidelines provided in Table 5. Since fluorescence from SYBR Green 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.

Table 5. General Guidelines for Design of Primers

Length	18–30 nucleotides
GC content	40–60%
T_m	For best results, use commercially available oligo-design software such as OLIGO 6 (oligo.net) or Web-based tools such as Primer3 (frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi)* to determine primer T_ms. Simplified formula for estimating melting temperature (T_m): T_m = 2°C x (number of [A+T]) + 4°C x (number of [G+C]) Whenever possible, design primer pairs with similar T_m values.
Sequence	■ 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. ■ Ensure the length of the PCR product is less than 200 bp.

Table continued on next page

* Rozen, S. and Skaletsky, H.J. (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz, S. and Misener, S., eds. Bioinformatics Methods and Protocols: Methods in Molecular Biology. Totowa, NJ: Humana Press, pp. 365–386.

Table 5. Continued

Sequence	■ Avoid complementarity of 2 or more 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 or Cs at the 3' end. ■ Avoid a 3'-end T. Primers with a T at the 3' end have a greater tolerance of mismatch. ■ Avoid complementary sequences within a primer sequence and between the primer pair. ■ Commercially available computer software (e.g., OLIGO 6) or Web-based tools (e.g., Primer3) can be used for primer design. Use the software to minimize the likelihood of formation of stable primer–dimers.
Special consider-ations for design of RT-PCR primers and probes	■ Design primers so that one half hybridizes to the 3' end of one exon and the other half to the 5' end of the adjacent exon (see Figure 1). The primers will therefore anneal to cDNA synthesized from spliced mRNAs, but not to genomic DNA, eliminating detection of contaminating DNA. ■ Alternatively, RT-PCR primers should be designed to flank a region that contains at least one intron. Products amplified from cDNA (no introns) will be smaller than those amplified from genomic DNA (containing introns). If possible, select a target with very long introns: the RNA target may then be preferentially amplified because of the higher PCR efficiency of this shorter PCR product without introns. If genomic DNA is detected (i.e., presence of amplification product in "No RT" control), treat the template RNA with RNase-free DNase, or synthesize cDNA using the QuantiTect Reverse Transcription Kit (which includes integrated genomic DNA removal). Alternatively, redesign primers to avoid amplification of genomic DNA.

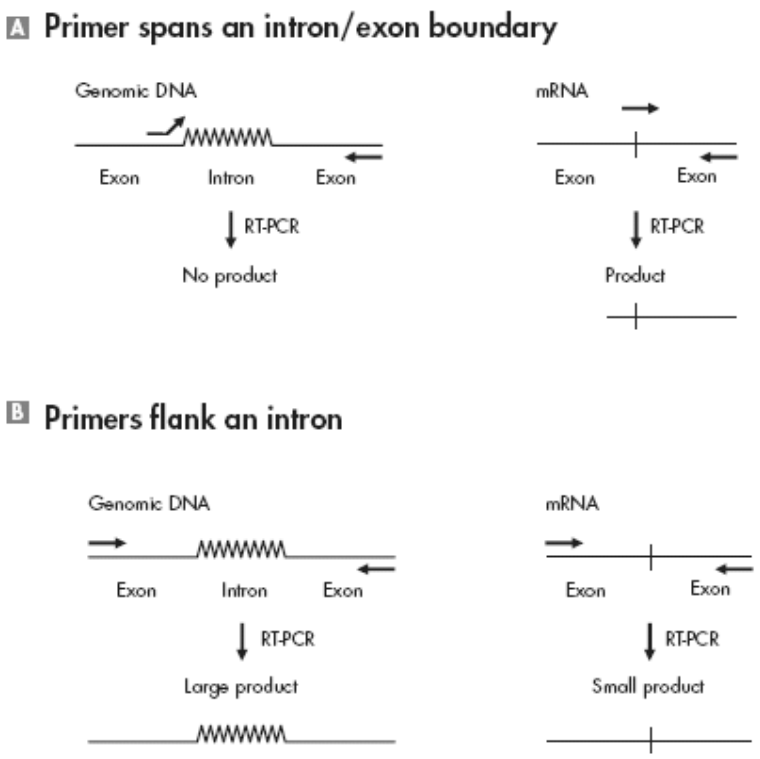

Figure 1. Primer design. Primer design to **A** eliminate or **B** detect amplification from contaminating genomic DNA.

Handling and storing primers

Guidelines for handling and storing primers are provided in Table 6 below. For optimal results, we recommend only combining primers of comparable quality.

Table 6. General Guidelines for Handling and Storing Primers

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 (10 mM Tris·Cl, 1 mM EDTA, pH 8.0) for standard primers.
Storage	Primers should be stored in TE 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, since they may lead to degradation.

Table continued on next page

Table 6. Continued

Dissolving primers	Before opening a tube containing lyophilized primer, spin the tube briefly to collect all material at the bottom of the tube. To dissolve the primer, add the required volume of TE, 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 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} = ε_{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: ε_{260} = 0.89 x [(A x 15,480) + (C x 7340) + (G x 11,760) + (T x 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 (see back cover) for a protocol.

Appendix C: Quantifying Gene Expression Levels and Generating Standard Curves

This appendix provides information on quantification of target nucleic acids. Further information can be found in *Critical Factors for Successful Real-Time PCR*. To obtain a copy, contact QIAGEN Technical Services, or visit www.qiagen.com/literature/defaultbrochures.aspx to download a PDF.

Absolute and relative quantification

Target nucleic acids can be quantified using either absolute quantification or relative quantification. Absolute quantification determines the absolute amount of a target (expressed as a copy number or concentration), whereas relative quantification determines the ratio between the amount of a target and the amount of a reference nucleic acid, usually a suitable housekeeping gene. This normalized value can then be used to compare, for example, differential gene expression in different samples.

Absolute quantification

The absolute amount of a target nucleic acid is determined using external standards. The sequence of the standards is usually the same as or very similar to the target sequence, but the primer binding sites of the standards must be identical to those in the target sequence. This ensures that both the standards and the target are amplified with equivalent efficiencies, which is essential for absolute quantification. A standard curve (plot of C_T value/crossing point against log of amount of standard) is generated using different dilutions of the standard. The target and each of the standards are amplified in separate tubes. The C_T value of the target is compared with the standard curve, allowing calculation of the initial amount of the target. It is important to select an appropriate standard for the type of nucleic acid to be quantified (see page 25).

Relative quantification

With this method, the amounts of the target genes and the reference gene within the same sample are determined, and ratios are calculated between each target gene and the reference gene. These normalized values can then be used to compare, for example, differential gene expression in different samples. The most common application of this method is analysis of gene expression or, more generally, determination of the abundance of RNA targets. The expression level of the reference gene, such as a housekeeping gene, must not vary under different experimental conditions, or in different states of the same tissue (e.g., “disease” versus “normal” samples). The level is therefore used as a reference value for quantification. The quantification procedure differs depending on whether the target genes and the reference gene are amplified

with comparable or different efficiencies. For determination of PCR efficiency, see below.

Different amplification efficiencies

The amplification efficiencies of target and reference genes are sometimes different due to differences in primer binding sites, PCR product sequences, and PCR product sizes. If this is the case, we recommend generating several standard curves (see below), one for each target or reference gene. The standards can be, for example, cDNA derived from total RNA prepared from a reference cell line. The amounts of the target genes and the reference gene are determined by comparing their C_T values with the corresponding standard curve. Ratios can then be calculated between each target gene and the reference gene. Since the expression of the reference gene remains the same between different samples, the ratio of the target genes to the reference gene will vary depending on the expression of the target genes (e.g., in different tissues).

Comparable amplification efficiencies

If the amplification efficiencies of the target and reference genes are the same, only the standard curve for the reference gene needs to be generated. The amounts of the target and reference genes are determined by comparing their C_T values with this standard curve.

Alternatively, the comparative or $\Delta\Delta C_T$ method can be used. This involves comparing C_T values, and does not require preparation of standard curves. This method can only be used if the amplification efficiencies of the target and reference genes are nearly equivalent.

Determination of PCR efficiency

To compare the amplification efficiencies of, for example, 2 target genes (targets A and B), prepare different dilutions each target. Amplify the different dilutions by real-time PCR or two-step RT-PCR. Subtract the C_T values of target A from the C_T values of target B. Plot the differences in C_T values against the logarithm of amount of target. If the slope of the resulting straight line is <0.1, the amplification efficiencies are comparable.

Generating standard curves

Standard curves can be used in both absolute and relative quantification. To generate a standard curve, at least 5 different amounts of the standard should be quantified, and the amount of unknown target should fall within the range of

the standard curve. Reactions should be carried out in at least triplicate, especially when quantifying standards of low copy number.

Standards

For absolute quantification of DNA and RNA molecules (see page 24), the copy number or concentration of the nucleic acids used as standards must be known. In addition, standards should show the following features:

- Primer binding sites identical to the target to be quantified
- Sequence between primer binding sites identical or highly similar to target sequence
- Sequences upstream and downstream from the amplified sequence identical or similar to "natural" target

RNA standards

For quantification of RNA, we strongly recommend using RNA molecules as standards. Depending on the sequence and structure of the target and the efficiency of reverse transcription, only a proportion of the target RNA will be reverse transcribed. The DNA generated during reverse transcription serves as the template for amplification in the subsequent PCR. The use of RNA standards takes the variable efficiency of the RT reaction into account.

RNA standards can be created by cloning part or all of the transcript of interest into a standard cloning vector. The insert can be generated by RT-PCR from total RNA or mRNA, or by PCR from cDNA. The cloning vector must contain an RNA polymerase promoter such as T7, SP6, or T3. Ensure that in vitro transcription of the insert leads to generation of the sense transcript. After in vitro transcription, plasmid DNA must be removed completely with RNase-free DNase, since residual plasmid DNA will lead to errors in spectrophotometric determination of RNA concentration and will also serve as a template in the subsequent PCR. Furthermore, ensure that the RNA used as a standard does not contain any degradation products or aberrant transcripts by checking that it migrates as a single band in gel electrophoresis.

After determination of RNA concentration by spectrophotometry, the copy number of standard RNA molecules can be calculated using the following formula:

(X g/µl RNA / [transcript length in nucleotides x 340]) x 6.022 x 10^{23} = Y molecules/µl

Example

Transcript length: 500 nucleotides

Concentration: 30 ng/µl = 30 x 10^{-9} g/µl

Calculation: $(30 \times 10^{-9}$ g/µl / [500 x 340]) x $6.022 \times 10^{23} = 1.1 \times 10^{11}$ molecules/µl

An alternative to the use of in vitro transcripts as RNA standards is the use of a defined RNA preparation (e.g., from a cell line or virus preparation), for which the absolute concentration of the target has already been determined.

DNA standards

Several types of DNA can be used as standards for the absolute quantification of DNA.

Plasmid DNA

The most convenient way to create a DNA standard is to clone a PCR product into a standard vector. Advantages of this method are that large amounts of standard can be produced, its identity can be verified by sequencing, and the DNA can easily be quantified by spectrophotometry. Plasmid standards should be linearized upstream or downstream of the target sequence, rather than using supercoiled plasmid for amplification. This is because the amplification efficiency of a linearized plasmid often differs from that of the supercoiled conformation and more closely simulates the amplification efficiency of genomic DNA or cDNA.

After spectrophotometric determination of plasmid DNA concentration, the copy number of standard DNA molecules can be calculated using the following formula:

(X g/µl DNA / [plasmid length in basepairs x 660]) x 6.022×10^{23} = Y molecules/µl

Example

Plasmid length: 3000 bp

Concentration: 100 ng/µl = 100×10^{-9} g/µl

Calculation: $(100 \times 10^{-9}$ g/µl / [3000 x 660]) x $6.022 \times 10^{23} = 3 \times 10^{10}$ molecules/µl

PCR fragment

A PCR product containing the target sequence can also be used as a DNA standard. We recommend including at least 20 bp upstream and downstream of the primer binding sites of the amplicons. Copy number is calculated using the formula for plasmid DNA (see above), replacing "plasmid length" with the length of the PCR product.

Genomic DNA

If the target of interest is present in only one copy per haploid genome and amplification of pseudogenes and/or closely related sequences can be excluded, genomic DNA can also be used as a DNA standard for absolute quantification. The copy number of the target present in the genomic DNA can be directly calculated if the genome size of the organism is known.

Example

Organism: *Mus musculus*

Genome size (haploid): 2.7×10^9 bp

Molecular weight: 1.78×10^{12} Daltons

1.78×10^{12} g of genomic DNA corresponds to 6.022×10^{23} copies of a single-copy gene.

1 µg of genomic DNA corresponds to 3.4×10^5 copies of a single-copy gene.

Appendix D: Controls

No template control (NTC)

All quantification experiments should include an NTC, containing all the components of the reaction except for the template. This enables detection of contamination.

RT control

All RT-PCR experiments should include a negative control to test for contaminating DNA. However, detection of this contamination can be eliminated by using suitable primers (see Table 5, pages 20). If it is not possible to use such primers, DNA contamination can be detected by performing a control reaction in which no reverse transcription is possible. The control RT reaction contains all components including template RNA, except for the reverse transcriptase enzyme. Reverse transcription therefore cannot take place. When an aliquot of this control is used as a template in PCR, the only template available is contaminating DNA.

Positive control

In some cases it may be necessary to include a positive control, containing a known concentration of template. This is usually a substitute for absolute standards and is used to test only for presence or absence of the target, but does not yield detailed quantitative information. Ensure that the positive control contains at least the minimum amount of DNA required for accurate detection.

Appendix E: Data Analysis

When carrying out data analysis, follow the recommendations provided by the manufacturer of your real-time cycler. Fundamental guidelines for data analysis and some important considerations are given below.

General considerations for data analysis

Real-time PCR data are produced as sigmoidal-shaped amplification plots (when using a linear scale), in which fluorescence is plotted against the number of cycles (Figure 2, page 30).

- The threshold cycle (C_T value) serves as a tool for calculation of the starting template amount in each sample. This is the cycle in which there is the first detectable significant increase in fluorescence.
- The optimal threshold setting depends on the reaction chemistries used for PCR. Therefore, an optimal threshold setting established for another kit may not be suitable for the QuantiFast SYBR Green PCR Kit, and may need to be adjusted.
- The method for determination of C_T values differs depending on the real-time cycler used. Check the handbook or the software help file for your real-time cycler for details on threshold settings.
- Whenever possible, select the option for automatic calculation of threshold and baseline for your real-time cycler. **However, note that the default values for data analysis in the cycler software will not always provide the most accurate results.**
- Most real-time cyclers contain a function that determines the noise level in early cycles, where there is no detectable increase in fluorescence due to PCR products (usually referred to as the baseline settings). Adjust the settings for this function.

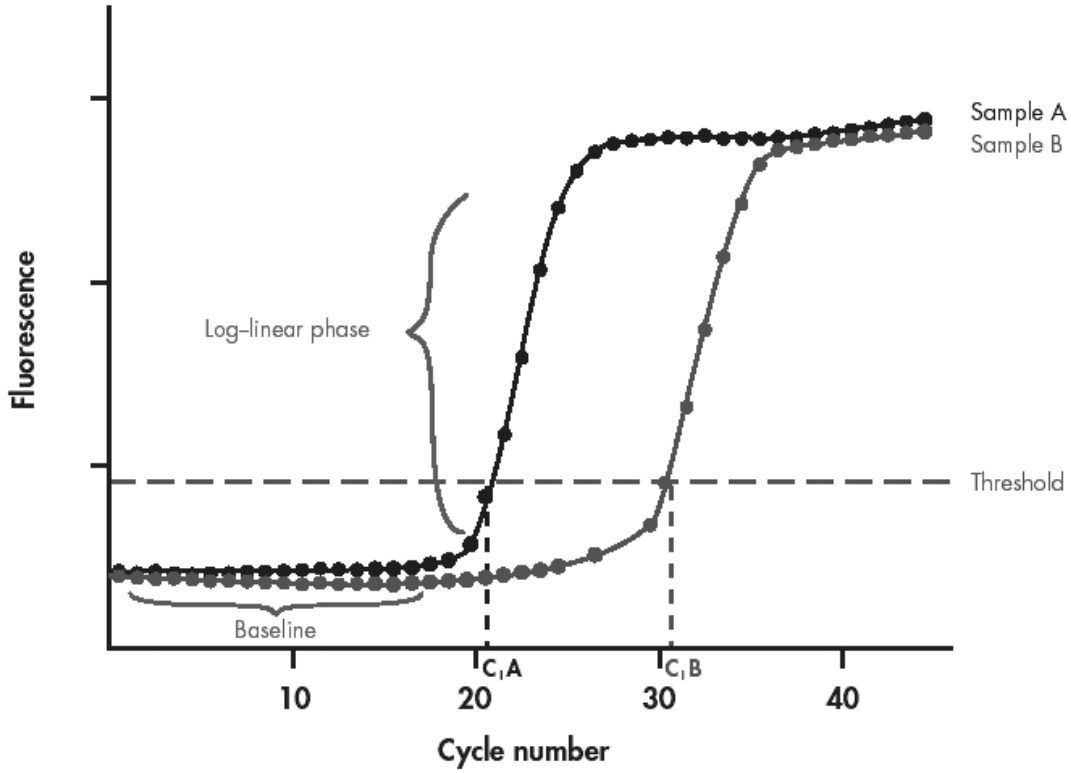

Figure 2. Typical amplification plot. Amplification plots showing increases in fluorescence from 2 samples (A and B). Sample A contains a higher amount of starting template than sample B.

Applied Biosystems instruments

Before performing data analysis on Applied Biosystems instruments, read the important points below. For further details, refer to the handbook, supplementary literature, or software help file for the instrument being used.

ABI PRISM 7900

The following points only apply to SDS software version 2.1 or higher. If you work with a version earlier than 2.1, we recommend updating your SDS software to the most recent version.

- The analysis settings for the baseline and threshold need to be set.
- Details on data analysis are available in the SDS 2.1 online help (from the "Help" menu, select "SDS Online Help" and enter a search term).
- If you observe wells with a C_T value that strongly deviates from those of replicate wells when using the automatic analysis settings, you should record the positions of these unusual wells and reanalyze the plate.

- If you observe problems using the automatic calculation option, you may configure the analysis settings manually. **Note that the default values for the analysis settings entered in the software will not always provide the most accurate results.**

ABI PRISM 7700

The following points only apply to SDS software version 1.7 or higher. If you work with a version earlier than 1.7, we recommend updating your SDS software to the most recent version.

- Check baseline and threshold settings.
- Analyze the plate. You may wish to save this setup in a separate file.
- Export the C_T values for the assays if you want to perform data analysis (e.g., using a spreadsheet program).
- **Note that the default values for the analysis settings entered in the software will not always provide the most accurate results.**

ABI PRISM 7000

The following points only apply to software version 1.1 or higher. If you work with a version earlier than 1.1, we recommend updating your SDS software to the most recent version.

- The analysis settings for the baseline and threshold need to be set.
- Details on data analysis are provided in the online help (from the "Help" menu, select "Contents and Index" and enter a search term).
- If you observe wells with a C_T value that strongly deviates from those of replicate wells, you should record the positions of these unusual wells and reanalyze the plate.
- If you observe problems using the automatic option, you may configure the analysis settings manually. **Note that the default values for the analysis settings entered in the software will not always provide the most accurate results.**

Applied Biosystems 7300

The following points only apply to software version 1.22 or higher. If you work with a version earlier than 1.22, we recommend updating your SDS software to the most recent version.

- The analysis settings for the baseline and threshold need to be set.
- Details on data analysis are provided in the online help (from the "Help" menu, select "Contents and Index" and enter a search term).

- If you observe wells with a C_T value that strongly deviates from those of replicate wells, you should record the positions of these unusual wells and reanalyze the plate.
- If you observe problems using the automatic option, you may configure the analysis settings manually. **Note that the default values for the analysis settings entered in the software will not always provide the most accurate results.**

Applied Biosystems 7500

The following points only apply to software version 1.22 or higher. If you work with a version earlier than 1.22, we recommend updating your SDS software to the most recent version.

- The analysis settings for the baseline and threshold need to be set.
- If you observe wells with a C_T value that strongly deviates from those of replicate wells, you should record the positions of these unusual wells and reanalyze the plate.
- If you observe problems using the automatic option, you may configure the analysis settings manually. **Note that the default values for the analysis settings entered in the software will not always provide the most accurate results.**
- Details on data analysis are provided in the online help (from the "Help" menu, select "Contents and Index" and enter a search term).

LightCycler system

There are 2 different methods of calculating crossing points: the fit point and the second derivative maximum method.

Fit point method: The principle of this method is the same as that used for the Applied Biosystems instruments. Use the arithmetic mode of baseline adjustment when analyzing data obtained with SYBR Green I.

Noise band: The noise band must be set according to the threshold in the log-linear phase of PCR.

Fit points: These are a defined number of reading points in the log-linear phase, used for calculation of a straight line that represents the linear portion of the amplification plot. The number of fit points can be changed by the user.

Crossing point: This is the cycle at which the straight line (calculated using fit points) crosses the noise band.

Second derivative maximum method: The point at which the maximal increase of fluorescence within the log-linear phase takes place is calculated by determining the second derivative maxima of the amplification curves. The software calculates at which cycle number this point is reached. It is not necessary to set a noise band.

Standard curves

Standard samples with known template amounts are defined in the "sample setup" view. The results from all wells defined as standards are used following the run for the generation of a standard curve. The C_Ts or crossing points are plotted against the log of the template amount, resulting in a straight line. C_T values for these samples and the standard curve are then used to calculate the amount of starting template in experimental samples.

Experiment report

The experiment report is a summary of the PCR results. At the end of experiments, sample names, template amounts, C_T values or crossing points, and standard deviations are listed.

Melting curves

All cyclers can perform a melting curve (except for the ABI PRISM 7700 with sequence detection software earlier than 1.7).

To carry out melting curve analysis, the temperature is increased very slowly from a low temperature (e.g., 65°C) to a high temperature (e.g., 95°C). At low temperatures, all PCR products are double stranded, so SYBR Green I binds to them and fluorescence is high, whereas at high temperatures, PCR products are denatured, resulting in rapid decreases in fluorescence.

The fluorescence is measured continuously as the temperature is increased and plotted against temperature. A curve is produced, because fluorescence decreases slightly through the lower end of the temperature range, but decreases much more rapidly at higher temperatures as the melting temperatures of nonspecific and specific PCR products are reached. The detection systems calculate the first derivatives of the curves, resulting in curves with peaks at the respective T_ms. Curves with peaks at a T_m lower than that of the specific PCR product indicate the formation of primer–dimers, while diverse peaks with different T_ms or plateaus indicate production of nonspecific products or a smear.

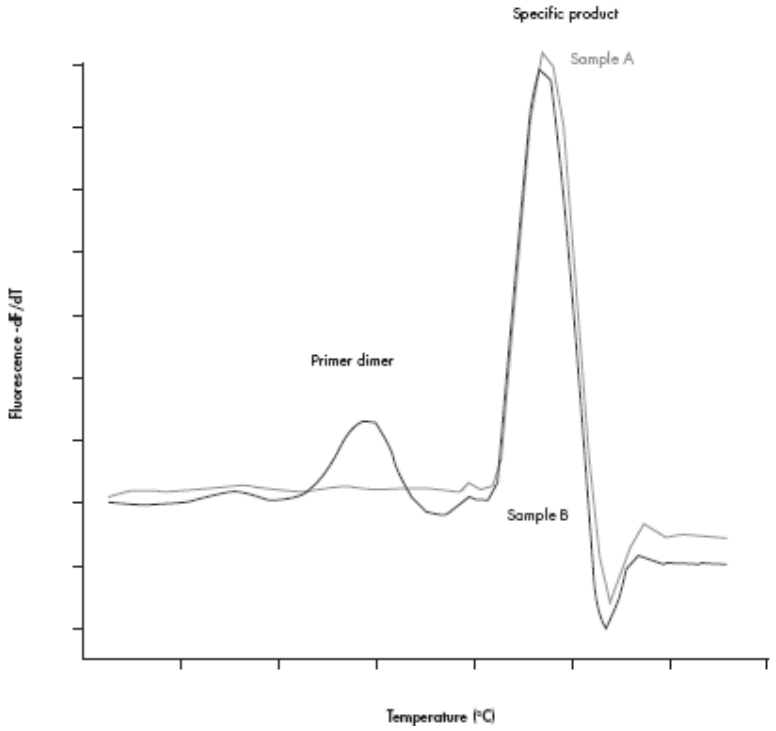

Figure 3. Melting curve analysis. Melting curve analysis of 2 samples (A and B). Sample A yields only 1 peak resulting from the specific amplification product (primer–dimers not coamplified). Sample B shows a peak from the specific product and a peak at a lower temperature from amplification of primer–dimers.

Appendix F: Collecting Well Factors on Bio-Rad iQ Cyclers

Bio-Rad iQ cyclers (e.g., iCycler iQ, iQ5, and MyiQ) need to collect well factors at the start of each real-time PCR experiment to compensate for any excitation or pipetting nonuniformity. When performing SYBR Green based real-time PCR, **dynamic well factors** cannot be collected from the experimental plate unless the PCR master mix has been spiked with fluorescein, an additional fluorophore. This is because SYBR Green fluoresces insufficiently in the initial PCR step, where there is insufficient double-stranded DNA to bind SYBR Green and allow fluorescence.

Alternatively, **external well factors** can be collected from an external well factor plate containing only fluorescein solution. In our experience, collecting external well factors is a more reliable and convenient alternative to collecting dynamic well factors when using QuantiFast SYBR Green Kits on Bio-Rad cyclers.

If using a QuantiFast SYBR Green Kit on the iCycler iQ system, follow the procedure below to prepare and run an external well factor plate.

Procedure

F1. Dilute 10x External Well Factor Solution (Bio-Rad, cat. no. 170-8794; contains fluorescein) to a 1x concentration with distilled water.

F2. Distribute the diluted solution into the wells of a PCR plate and seal with optically clear sealing film.

The volume of diluted solution per well depends on the real-time PCR volume. For example, if the PCR volume will be 25 µl, then distribute 25 µl of diluted solution per well.

F3. Briefly centrifuge the external well factor plate, place it into the Bio-Rad iQ cycler, and close the lid.

F4. Select the SYBR Green thermal protocol and plate setup files, and click "Run with selected Protocol".

F5. In the "RunPrep" screen, select External Plate as "Well Factor" and click "Begin Run".

The iCycler iQ system automatically inserts a 3-cycle protocol, **External.tmo** in front of your thermal protocol to collect optical data.

F6. After well factors are calculated, the Bio-Rad iQ cycler pauses. Replace the external well factor plate with your experimental plate. Click "Continue Running Protocol" to start your experiment.

Note: Once the external well factor plate is prepared, it can be reused several times (over 250 times) until the iCycler iQ system indicates that the fluorophore intensity is insufficient to calculate well factors. Store the external well factor plate at –20°C between experiments, and thaw and centrifuge it before use. Be sure to protect the plate from exposure to light when not in use.

Ordering Information

Product	Contents	Cat. no.
QuantiFast SYBR Green PCR Kit (400)	For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix (contains ROX dye), 2 x 1.9 ml RNase-Free Water	204054
QuantiFast SYBR Green PCR Kit (2000)	For 2000 x 25 µl reactions: 25 ml 2x Master Mix (contains ROX dye), 20 ml RNase-Free Water	204056
Accessories		
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
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
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
Related products		
QuantiFast SYBR Green RT-PCR Kit — for fast, quantitative, real-time, one-step RT-PCR using SYBR Green I		
QuantiFast SYBR Green RT-PCR Kit (400)	For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix (contains ROX dye), 100 µl RT Mix, 2 x 1.9 ml RNase-Free Water	204154
QuantiFast SYBR Green RT-PCR Kit (2000)	For 2000 x 25 µl reactions: 25 ml 2x Master Mix (contains ROX dye), 0.5 ml RT Mix, 20 ml RNase-Free Water	204156

Product	Contents	Cat. no.
QuantiFast Probe PCR Kits — for fast, quantitative, real-time PCR and two-step RT-PCR using sequence-specific probes		
For all instruments from Applied Biosystems except the Applied Biosystems 7500:		
QuantiFast Probe PCR Kit (400)	For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix (contains ROX dye), 2 x 1.9 ml RNase-Free Water	204254
QuantiFast Probe PCR Kit (2000)	For 2000 x 25 µl reactions: 25 ml 2x Master Mix (contains ROX dye), 20 ml RNase-Free Water	204256
For the Applied Biosystems 7500 and instruments from Bio-Rad, Cepheid, QIAGEN, Eppendorf, Roche, and Agilent:		
QuantiFast Probe PCR +ROX Vial Kit (400)	For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix (without ROX dye), 210 µl ROX Dye Solution, 2 x 1.9 ml RNase-Free Water	204354
QuantiFast Probe PCR +ROX Vial Kit (2000)	For 2000 x 25 µl reactions: 25 ml 2x Master Mix (without ROX dye), 1.05 ml ROX Dye Solution, 20 ml RNase-Free Water	204356
QuantiFast Probe RT-PCR Kits — for fast, quantitative, real-time, one-step RT-PCR using sequence-specific probes		
For all instruments from Applied Biosystems except the Applied Biosystems 7500:		
QuantiFast Probe RT-PCR Kit (400)	For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix (contains ROX dye), 100 µl RT Mix, 2 x 1.9 ml RNase-Free Water	204454
QuantiFast Probe RT-PCR Kit (2000)	For 2000 x 25 µl reactions: 25 ml 2x Master Mix (contains ROX dye), 0.5 ml RT Mix, 20 ml RNase-Free Water	204456

Product	Contents	Cat. no.
For the Applied Biosystems 7500 and instruments from Bio-Rad, Cepheid, QIAGEN, Eppendorf, Roche, and Agilent:		
QuantiFast Probe RT-PCR +ROX Vial Kit (400)	For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix (without ROX dye), 210 µl ROX Dye Solution, 100 µl RT Mix, 1.9 ml RNase-Free Water	204554
QuantiFast Probe RT-PCR +ROX Vial Kit (2000)	For 2000 x 25 µl reactions: 25 ml 2x Master Mix (without ROX dye), 1.05 ml ROX Dye Solution, 0.5 ml RT Mix, 20 ml RNase-Free Water	204556
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
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

* Other kit sizes and formats available; please inquire.

* Other kit sizes and formats available; please inquire.

Product	Contents	Cat. no.
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
RNeasy Protect Bacteria Mini Kit — for in vivo stabilization of the gene expression profile in bacteria and subsequent RNA purification		
RNeasy Protect Bacteria Mini Kit (50)†	RNeasy Mini Kit (50) and RNAprotect® Bacteria Reagent (2 x 100 ml)	74524
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

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.

Visit www.qiagen.com/geneXpression to find out more about standardized solutions for gene expression analysis — from RNA preparation to real-time RT-PCR

† Other kit format available; please inquire.

‡ Additional purification of protein is possible using a supplementary protocol; for details, visit www.qiagen.com/goto/AllPrepDR.

Notes

Notes

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