QuantiTect™ SYBR® Green PCR Handbook

For quantitative, real-time PCR and two-step RT-PCR

March 2002

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Contents

Kit Contents

QuantiTect™ SYBR® Green PCR Kit	
Catalog Number	**204143**
No. of 50 µl reactions	**200**
2x QuantiTect SYBR Green PCR Master Mix, containing:	3 x 1.7 ml
• HotStarTaq™ DNA Polymerase	
• QuantiTect SYBR Green PCR Buffer	
• dNTP mix including dUTP	
• SYBR Green I	
• ROX (passive reference dye)	
• 5 mM $MgCl_2$	
RNase-free water	2 x 2.0 ml
Handbook	1

Shipping and Storage Conditions

The QuantiTect SYBR Green PCR Kit for quantitative, real-time PCR and two-step RT-PCR is shipped on dry ice. It should be stored immediately upon receipt at –20°C in a constant-temperature freezer and protected from light. When stored under these conditions and handled correctly, the products can be kept at least until the expiration date without showing any reduction in performance. The 2x QuantiTect SYBR Green PCR Master Mix can also be stored at 2–8°C for up to 6 months without showing any reduction in performance.

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 QuantiTect 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 call one of the QIAGEN Technical Service Departments or local distributors (see inside front cover).

Product Use Limitations

The QuantiTect SYBR Green PCR Kit is designed, developed, and sold for research purposes only. It is not to be used for human diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this text.

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 Services Department or distributor. We will credit your account or exchange the product — as you wish.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/ts/msds.asp** where you can find, view, and print the MSDS for this kit and its components.

24-hour emergency information

Emergency medical information can be obtained 24 hours a day from:
Poison Information Center Mainz, Germany
Tel: +49-6131-19240

Introduction

The QIAGEN QuantiTect SYBR Green PCR Kit provides accurate real-time quantification of DNA and cDNA targets in an easy-to-handle format. The fluorescent dye SYBR Green I in the master mix enables rapid analysis of many different targets without having to synthesize target-specific labeled probes. High specificity and sensitivity in PCR is achieved by the use of the hot-start enzyme, HotStarTaq DNA Polymerase, together with a specialized buffer. The kit has been optimized for use with any real-time cycler, such as the ABI™ Sequence Detection Systems (e.g., ABI PRISM® 7700), the LightCycler® (Roche, Idaho Technologies), the iCycler™ (Bio-Rad), or the DNA Engine Opticon™ (MJ Research) systems. This handbook describes generalized protocols for use with these systems.

HotStarTaq DNA Polymerase

The QuantiTect SYBR Green PCR Master Mix contains HotStarTaq Polymerase, which is a modified form of QIAGEN *Taq* DNA Polymerase. HotStarTaq DNA Polymerase is provided in an inactive state and has no enzymatic activity at ambient temperatures. 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 by a 15-minute, 95°C incubation step, which is easily incorporated into existing thermal cycling programs. The hot start enables reactions to be set up at room temperature, which is rapid and convenient.

QuantiTect SYBR Green PCR Buffer

The QuantiTect SYBR Green PCR Buffer is based on the unique QIAGEN PCR buffer system and has been specifically adapted for quantitative PCR analysis using the fluorescent dye SYBR Green I. It 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 usually not required.

SYBR Green I

The QuantiTect SYBR Green PCR Master Mix contains an optimized concentration of the fluorescent dye SYBR Green I. SYBR Green I binds all double-stranded DNA molecules, emitting a fluorescent signal on binding. The QuantiTect SYBR Green PCR Kit can therefore be used for quantification of different targets without requiring sequence-specific fluorescent probes. The QuantiTect SYBR Green PCR Master Mix can be stored at 2–8°C or –20°C without loss of SYBR Green I 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

The fluorescent dye ROX included in the master mix serves as an internal reference for normalization of the SYBR Green I fluorescent signal when carrying out reactions using the ABI Sequence Detection Systems. It allows correction of well-to-well variation due to pipetting inaccuracies and fluorescence fluctuations. However, its presence does not interfere with reactions performed using the LightCycler, iCycler, or DNA Engine Opticon Systems, since it is not involved in PCR and has an emission spectrum completely different from that of SYBR Green I.

Use of uracil-N-glycosylase (UNG)

The 2x QuantiTect SYBR Green PCR Master Mix contains dUTP, which partially replaces dTTP. The QuantiTect SYBR Green PCR Kit therefore allows the optional use of a uracil-N-glycosylase (UNG) pretreatment of the reaction, which can be used if contamination with carried-over PCR products is suspected.

Product Description

2x QuantiTect SYBR Green PCR Master Mix contains:

HotStarTaq DNA Polymerase:	HotStarTaq DNA Polymerase is a modified form of a recombinant 94 kDa DNA polymerase, originally isolated from *Thermus aquaticus*, cloned into *E. coli*. (Deoxynucleoside-triphosphate: DNA deoxynucleotidyltransferase, EC 2.7.7.7).
QuantiTect SYBR Green PCR Buffer:	Contains Tris·Cl, KCl, $(NH_4)_2SO_4$, 5 mM $MgCl_2$, pH 8.7 (20°C)
dNTP mix:	Contains dATP, dCTP, dGTP, and dTTP/dUTP; ultrapure quality
Fluorescent dyes:	SYBR Green I and ROX
RNase-free water:	Ultrapure quality, PCR-grade

Quality Control

QuantiTect SYBR Green PCR Master Mix	(See quality-control label inside kit lid for lot-specific values)
PCR sensitivity and reproducibility assay:	Sensitivity and reproducibility in real-time PCR are tested in parallel 50 µl reactions containing 10-fold dilutions of nucleic acid template.
HotStarTaq DNA Polymerase	(included in QuantiTect SBYR Green PCR Master Mix)
	Efficiency and reproducibility in PCR are tested. Functional absence of exonucleases, endonucleases, and proteases is tested.
Buffers and reagents	(included in QuantiTect SYBR Green PCR Master Mix)
QuantiTect SYBR Green PCR Buffer:	Conductivity and pH are tested.
RNase-free water:	Conductivity, pH, and RNase activities are tested.

Protocol for Quantitative, Real-Time PCR Using ABI Sequence Detection Systems and Other Real-Time Thermal Cyclers

This protocol is intended for use with ABI Sequence Detection Systems (e.g., ABI PRISM 7700), iCycler, or DNA Engine Opticon systems. For quantitative, real-time PCR on the LightCycler system, see page 13.

! IMPORTANT

- For the highest efficiency in real-time PCR using SYBR Green, targets should ideally be **100–150 bp in length**.
- The PCR must start with an **initial incubation step of 15 minutes at 95°C** in order to activate the HotStarTaq DNA Polymerase.
- Always start with an **initial Mg^{2+} concentration of 2.5 mM**, as provided in the 2x QuantiTect SYBR Green PCR Master Mix.

Protocol

1. **Thaw 2x QuantiTect SYBR Green PCR Master Mix (if stored at –20°C), template DNA, primers, and RNase-free water. Mix the individual solutions.**

2. **Prepare a master 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 cycler.

   Note: We strongly recommend starting with an initial Mg^{2+} concentration of 2.5 mM, as provided in the 2x QuantiTect SYBR Green PCR Master Mix. For a few targets, reactions may be improved by using Mg^{2+} concentrations up to 5 mM.

Table 1. Reaction components

Component	Volume/reaction	Final concentration
2x QuantiTect SYBR Green PCR Master Mix*	25 µl	1x
Primer A	Variable	0.3 µM†
Primer B	Variable	0.3 µM†
RNase-free water	Variable	–
Optional: Uracil-N-glycosylase	Variable	0.5 unit/reaction
Template DNA (added at step 4)	Variable	≤500 ng/reaction
Total volume	**50 µl**	

* *Provides a final concentration of 2.5 mM $MgCl_2$*

† *A final primer concentration of 0.3 µM is usually optimal. However, for individual determination of best concentration, a primer titration from 0.2 µM to 1.0 µM can be performed.*

3. **Mix the master mix thoroughly and dispense appropriate volumes into PCR tubes or plates.**

4. **Add template DNA (≤500 ng/reaction) to the individual PCR tubes containing the master mix.**

   For two-step RT-PCR, the volume of the cDNA added (from the RT reaction) as template should not exceed 10% of the final PCR volume.

5. **Program the ABI PRISM 7700/ABI GeneAmp 5700/iCycler/DNA Engine Opticon according to the program outlined in Table 2.**

   Data acquisition should be performed during the extension step. After performing a melting curve analysis (see step 7), an additional data acquisition step for further runs with the same target can be integrated (for details, see steps 8 and 9).

Table 2. Real-time cycler conditions

Step	Time	Temperature	Additional comments
UNG (optional) Carryover prevention	2 min	50°C	UNG will eliminate any dUMP-containing PCR products resulting from carryover contamination.
PCR			
Initial activation step	**15 min**	**95°C**	HotStarTaq DNA Polymerase is activated by this heating step.
3 (4)-step cycling:			
Denaturation	15 s	94°C	
Annealing	30 s	50–60°C	Approximately 5 to 8°C below T_m of primers
Extension	30 s	72°C	Perform fluorescence data collection, unless an additional data acquisition step has been integrated.
Optional: Data acquisition	15 s	x°C	T_m dimer < x < T_m product: see steps 8 and 9 for details
Cycle number	35–45 cycles		Cycle number depends on the amount of template DNA.

6. **Place the PCR tubes in the thermal cycler and start the cycling program.**
7. **Perform a melting curve analysis of the PCR product(s).**

   We highly recommend routinely performing this analysis step in order to verify specificity and identity of the PCR products. Melting curve analysis is an analysis step built into the software of real-time cyclers. Please follow instructions provided by the supplier. Generally, melting curve data between 65°C and 95°C should be acquired.

   Note: The T_m of a PCR product depends on buffer composition and salt concentration. T_m values obtained when using the QuantiTect SYBR Green reagents may differ from those obtained using other reagents.

   Depending on primer design and copy number of target, primer-dimers may occur. These can be distinguished from the specific product through their lower melting point.
8. **Optional: Repeat the previous run, including an additional data acquisition step.**

   In order to suppress fluorescence readings caused by the generation of primer-dimers, an additional data acquisition step can be added to the 3-step cycling protocol (see Table 2). The temperature should be above the T_m of primer-dimers but approximately 3°C below the T_m of the specific PCR product. This method can increase the dynamic range and reliability of quantification by several orders of magnitude if primer-dimers are co-amplified.
9. **Optional: Check the specificity of PCR products by agarose gel electrophoresis.**

Protocol for Quantitative, Real-Time PCR Using the LightCycler System

! IMPORTANT

- Always start with the cycling conditions specified in this protocol, even if using previously established primer–template systems. Pay particular attention to Table 4 and step 7.
- For the highest efficiency in real-time PCR using SYBR Green, targets should ideally be **100–150 bp in length**.
- The PCR must start with an **initial incubation step of 15 minutes at 95°C** in order to activate the HotStarTaq DNA Polymerase.
- Always start with an **initial Mg^{2+} concentration of 2.5 mM**, as provided in the 2x QuantiTect SYBR Green PCR Master Mix.

Protocol LightCycler

Protocol

1. **Thaw 2x QuantiTect SYBR Green PCR Master Mix (if stored at –20°C), template DNA, primers, and RNase-free water. Mix the individual solutions.**
2. **Prepare a master 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 cycler.

   Note: We strongly recommend starting with an initial Mg^{2+} concentration of 2.5 mM, as provided in the 2x QuantiTect SYBR Green PCR Master Mix. For a few targets, reactions may be improved by using Mg^{2+} concentrations up to 5 mM.

Table 3. Reaction components using the LightCycler system

Component	Volume/reaction	Final concentration
2x QuantiTect SYBR Green PCR Master Mix*	10 µl	1x
Primer A	Variable	0.5 µM†
Primer B	Variable	0.5 µM†
RNase-free water	Variable	–
Optional: Uracil-N-glycosylase	Variable	0.5 unit/reaction
Template DNA (added at step 4)	Variable	≤1 µg/reaction
Total volume	**20 µl**	

* *Provides a final concentration of 2.5 mM $MgCl_2$*

† *A final primer concentration of 0.5 µM is usually optimal. However, for individual determination of best concentration, a primer titration from 0.5 µM to 1.0 µM can be performed.*

3. **Mix the master mix thoroughly and dispense appropriate volumes into PCR capillaries.**
4. **Add template DNA (≤ 1 µg/reaction) to the individual PCR capillaries.**

   For two-step RT-PCR, the volume of the cDNA added (from the RT reaction) as template should not exceed 10% of the final PCR volume.
5. **Program the LightCycler according to the program outlined in Table 4. Set fluorescence gains as described in Table 5 (for LightCycler software versions earlier than 3.5).**

   Data acquisition should be performed during the extension step. After performing a melting curve analysis (see step 7), an additional data acquisition step for further runs with the same target can be integrated (for details, see steps 8 and 9).

Table 4. Real-time cycler conditions for the LightCycler system

Step	Time	Temperature	Ramp	Additional comments
UNG (optional) Carryover prevention	2 min	50°C	20°C/s	UNG will eliminate any dUMP-containing PCR products resulting from carryover contamination.
PCR				
Initial activation step	**15 min**	**95°C**	**20°C/s**	HotStarTaq DNA Polymerase is activated by this heating step.
3 (4)-step cycling:				
Denaturation	15 s	94°C	20°C/s	
Annealing	20–30 s	50–60°C	20°C/s	Approximately 5 to 8°C below T_m of primers
Extension	10–30 s	72°C	20°C/s	Perform fluorescence data collection, unless an additional data acquisition step has been integrated. Extension time depends on product length. Allow 5 s per 100 bp, with a minimum extension time of 10 s.
Optional: Data acquisition	5 s	x°C	20°C/s	T_m dimer < x < T_m product: see steps 8 and 9 for details
Cycle number	35–55 cycles			Cycle number depends on the amount of template DNA.

Table 5. Fluorescence parameters for the LightCycler

Fluorimeter gain	Value
Channel 1 (F1)	15
Channel 2 (F2)	10
Channel 3 (F3)	10

Display mode: fluorescence channel 1/1 (F1/1)

LightCycler software versions 3.5 or later automatically adapt the fluorimeter gains for the fluorescence channels. No user-defined setting is required.

6. **Place the PCR capillaries in the LightCycler and start the cycling program.**
7. **Perform a melting curve analysis of PCR product(s).**

   We highly recommend routinely performing this analysis step in order to verify specificity and identity of the PCR products. Melting curve analysis is an analysis step built into the software of the LightCycler. Please follow instructions provided by the supplier. Generally, melting curve data between 65°C and 95°C should be acquired.

   Note: The T_m of a PCR product depends on buffer composition and salt concentration. T_m values obtained when using the QuantiTect SYBR Green reagents may differ from those obtained using other reagents.

   Depending on primer design and copy number of target, primer-dimers may occur. These can be distinguished from the specific product through their lower melting point.
8. **Optional: Repeat the previous run, including an additional data acquisition step.**

   In order to suppress fluorescence readings caused by the generation of primer-dimers, an additional data acquisition step can be added to the 3-step cycling protocol (see Table 4). The temperature should be above the T_m of primer-dimers but approximately 3°C below the T_m of the specific PCR product. This method can increase the dynamic range and reliability of quantification by several orders of magnitude if primer dimers are co-amplified.
9. **Optional: Check the specificity of PCR products by agarose gel electrophoresis.**

Troubleshooting Guide

This troubleshooting guide has been designed to provide a convenient optimization procedure for quantitative, real-time PCR and two-step RT-PCR using the QuantiTect SYBR Green PCR Kit. Troubleshooting hints are applicable to reactions carried out on any real-time cycler except where indicated otherwise. The scientists in QIAGEN Technical Services are happy to answer any questions you may have either about the information and protocols in this handbook, or about molecular biology applications in general (see inside front cover for contact information).

		Comments and suggestions
No product, or product detected late in PCR, or only primer-dimers detected		
1.	PCR annealing time too short	Use the recommended annealing time: **ABI Sequence Detection Systems, iCycler, and DNA Engine Opticon:** Annealing time 30 seconds. **LightCycler:** Annealing time 20–30 seconds.
2.	Extension time too short	Always use the extension times specified in the protocols. **ABI Sequence Detection Systems, iCycler, and DNA Engine Opticon:** Extension time should be 30 seconds for PCR products up to 500 bp. **LightCycler:** Extension time should be 5 seconds per 100 bp of PCR product, with a minimum extension time of 10 seconds.
3.	Mg^{2+} concentration not optimal	Always start with the Mg^{2+} concentration provided in the QuantiTect SYBR Green PCR Master Mix (2.5 mM final concentration). For a few targets only, an increase up to 5 mM Mg^{2+} may be helpful. Perform titration in 0.5 mM steps.
4.	Pipetting error or missing reagent	Check the concentrations and storage conditions of reagents, including primers and template nucleic acids. Repeat the PCR.
5.	HotStarTaq DNA Polymerase not activated	Ensure that the cycling program included the HotStarTaq DNA Polymerase activation step (15 minutes at 95°C) as described in step 5 of the protocols (pages 11 and 14).
6.	PCR product too long	For optimal results, PCR products should be between 100–150 bp in length and should not exceed 500 bp.
7.	PCR annealing temperature too high	Decrease annealing temperature in 3°C steps.

		Comments and suggestions
8.	Primer design not optimal	Check for presence of PCR products by melting curve (see page 32) or gel electrophoresis analysis. If no specific PCR products are detected, review the primer design guidelines (page 22).
9.	Primer concentration not optimal	Use optimal primer concentrations: **ABI Sequence Detection Systems, iCycler, or DNA Engine Opticon:** 0.3 µM each primer. **LightCycler:** 0.5 µM each primer.
10.	Problems with starting template	Check the concentration, storage conditions, and quality of the starting template (see Appendix, page 20). If necessary, make new serial dilutions of template nucleic acid from stock solutions. Repeat the PCR using the new dilutions.
11.	Insufficient starting template	Increase the template amount, if possible.
12.	Insufficient number of cycles	Increase the number of cycles.
13.	No detection activated	Check that fluorescence detection was activated in the cycling program.
14.	Wrong detection step	Ensure that fluorescence detection takes place during the extension step of the PCR program.
15.	UNG treatment combined with low annealing temperature	If annealing temperatures below 55°C are necessary for successful PCR, the optional UNG treatment should be performed using heat-labile UNG only.
16.	Primers degraded	Check for possible degradation of primers on a denaturing polyacrylamide gel.
17.	**RT-PCR only:** Volumes of RT reaction added were too high	High volumes of RT reaction added to PCR may reduce amplification efficiency. Generally the volume of reverse transcriptase reaction added should not exceed 10% of the final PCR volume.
18.	**Optional data acquisition step only:** Detection temperature too high	Ensure that the detection temperature is at least 3°C lower than the T_m of the specific product. When establishing a new primer–template system, always perform a 3-step cycling reaction first, without the optional data aquisition step (step 8, pages 12 and 15).

	Comments and suggestions
ABI Sequence Detection Systems and iCycler only:	
19. Wrong dye layer/filter chosen	Ensure that "SYBR Green" layer/filter is activated.
LightCycler only:	
20. Chosen fluorescence gains are 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	
1. Mg^{2+} concentration not optimal	Always start with the Mg^{2+} concentration provided in the QuantiTect SYBR Green PCR Master Mix (2.5 mM final concentration). For a few targets, an increase up to 5 mM Mg^{2+} may be helpful. Perform titration in 0.5 mM steps.
2. PCR annealing temperature too low	Increase annealing temperature in increments of 2°C.
3. Primer design not optimal	Review primer design (see page 22). If redesigning the primers is not possible, include additional data aquisition step above the T_m of primer-dimers (see step 5 in the protocols and the Appendix, page 34).
4. PCR product too long	For optimal results, PCR products should be between 100–150 bp in length and should not exceed 500 bp.
5. Primer-dimers co-amplified	Include an additional data aquisition step in the cycling program as indicated in the protocols (step 8, pages 12 and 15) to avoid the detection of primer-dimers.
6. Primers degraded	Check for possible degradation of primers on a denaturing polyacrylamide gel.
7. RT-PCR only: contamination with genomic DNA	Pretreat starting RNA template with DNase I. Alternatively, use primers located at splice junctions of the target mRNA to avoid amplification from genomic DNA.
No linearity in ratio of C_T value/crossing point to log of the template amount	
1. Template amount too high	Do not exceed maximum recommended amounts of template. For genomic DNA: **ABI Sequence Detection Systems, iCycler, and DNA Engine Opticon:** Do not use more than 500 ng template. **LightCycler:** Do not use more than 1 µg template.

2.	Template amount too low	Increase template amount, if possible.
3.	Primer-dimers co-amplified	Include an additional data aquisition step in the cycling program as indicated in the protocols (step 8, pages 12 and 15) to avoid the detection of primer-dimers.
4.	RT-PCR only: Volumes of RT reaction added were too high	High volumes of RT reaction added to PCR may reduce amplification efficiency. Generally the volume of reverse transcriptase reaction added should not exceed 10% of the final PCR volume.

High fluorescence in "No Template" control

1.	Contamination of reagents	Discard reaction components and repeat with new reagents.
2.	Contamination during reaction setup	Take appropriate safety precautions (e.g., use filter tips). Use uracil-N glycosylase to prevent carryover from previous reactions.

High fluorescence in "No RT" control reactions (RT-PCR only)

1.	Contaminating genomic DNA in RNA preparation	Design exon-spanning primers and/or probes to amplify/detect only the cDNA target. DNase digest the RNA starting template.

Varying fluorescence intensity

1.	Real-time cycler contaminated	Decontaminate the real-time cycler according to the supplier's instructions.
2.	Real-time cycler no longer calibrated	Recalibrate the real-time cycler according to the supplier's instructions.

ABI Sequence Detection Systems and iCycler only:

3.	Wavy curve at high template amounts	Reduce number of cycles used for baseline calculation.

LightCycler only:

4.	PCR mix not in capillary tip	Centrifuge capillary to bring PCR mix into capillary tip.
5.	Capillary not pushed down completely	Ensure that capillary is pushed down completely in the LightCycler carousel.
6.	Wrong detection channel	Make sure that Channel 1 is chosen.

Appendix

1. Starting template

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, EDTA, and other chemical solvents 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 the QIAprep® System for rapid plasmid purification, the QIAamp® and DNeasy® Systems for rapid purification of genomic DNA and viral nucleic acids, the RNeasy® System for preparation of RNA from various sources, and the Oligotex® System for mRNA isolation. For more information about these products, call one of the QIAGEN Technical Service Departments or local distributors (see inside front cover).

Determining concentration and purity of nucleic acids

The concentration of DNA and RNA should be determined by measuring the absorption 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 6 and 7.

Table 6. 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 7. 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 page 25).

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.

* *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 isolated using QIAGEN systems, 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 the nucleic acid to the tube walls, which would reduce the concentration of nucleic acid in solution.

2. Primer design, concentration, and storage

Prerequisites for successful PCR include design of optimal primer pairs, use of appropriate primer concentrations, and correct storage of primer solutions. Guidelines are provided in Table 8. 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.

Table 8. General guidelines for PCR primers

Length	18–30 nucleotides
GC content	40–60%
T_m	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. Optimal annealing temperatures may be above or below the estimated T_m. As a starting point, use an annealing temperature 5°C below T_m.
Sequence	• Ideally, the length of the PCR product is 100–150 bp. • Avoid complementarity of 2 or more bases at the 3' ends of primer pairs to reduce primer-dimer formation. • Avoid mismatches between the 3' end of the primer and the target-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.

Table continued overleaf

Table 8. Continued

	• Commercially available computer software (e.g., Primer Designer 1.0, Scientific Software, 1990; Oligo, Rychlik and Rhoads, 1989) can be used for primer design. Use the software to minimize the likelihood of formation of stable primer-dimers.
Special considerations for design of RT-PCR primers	• Design primers so that one half of the primer hybridizes to the 3' end of one exon and the other half to the 5' end of the adjacent exon (see Figure 1A, page 25). Primers will anneal to cDNA synthesized from spliced mRNAs, but not to genomic DNA. Thus, amplification of contaminating DNA is eliminated. • Alternatively, RT-PCR primers should be designed to flank a region that contains at least one intron (see Figure 1B, page 24). Products amplified from cDNA (no introns) will be smaller than those amplified from genomic DNA (containing introns). Size difference in products is used to detect the presence of contaminating DNA using melting curve analysis. If genomic DNA is detected, first treat the template RNA with RNase-free DNase, or if this is not possible, redesign primers to avoid amplification of genomic DNA.
Concentration	• Spectrophotometric conversion for primers: 1 A_{260} unit = 20–30 μg/ml • Molar conversions:

Primer length	pmol/μg	10 pmol*	15 pmol†
18mer	168	59 ng	89 ng
20mer	152	66 ng	99 ng
25mer	121	83 ng	124 ng
30mer	101	99 ng	149 ng

* *Final concentration 0.5 μM in 20 μl reaction*
† *Final concentration 0.3 μM in 50 μl reaction*

	• Depending on the real-time cycler used, optimal primer concentrations may vary as indicated in the protocols.
Storage	Lyophilized primers should be dissolved in a small volume of TE to make a concentrated stock solution. Prepare small aliquots of working solutions containing 10 pmol/μl to avoid repeated thawing and freezing. Store all primer solutions at –20°C. Primer quality can be checked on a denaturing polyacrylamide gel; a single band should be seen.

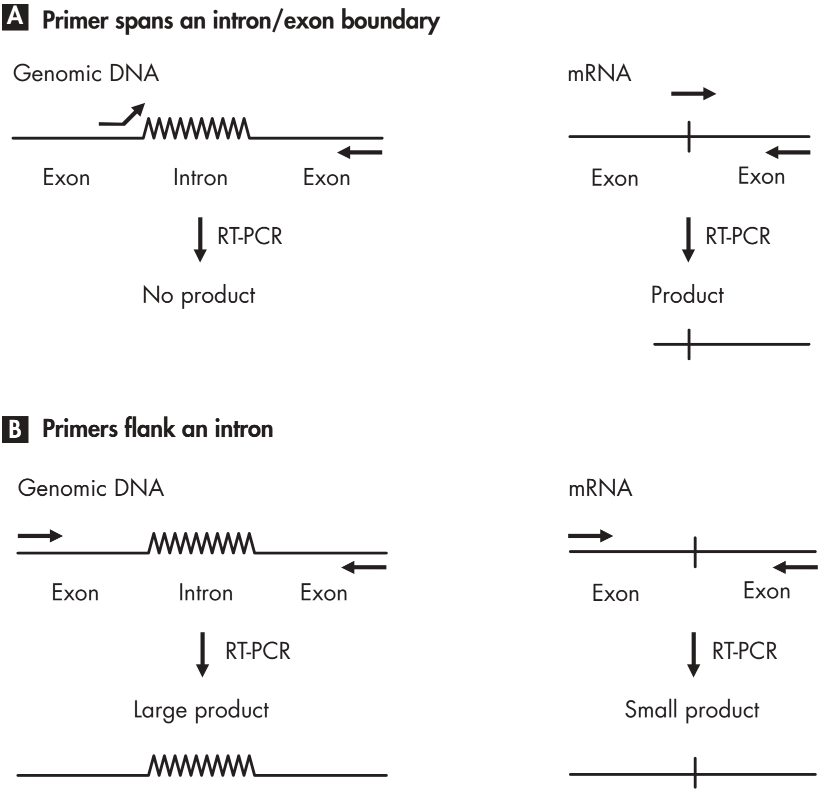

Figure 1. *Primer design to* **A** *eliminate or* **B** *detect amplification from contaminating genomic DNA*

3. Number of cycles

A cycling program usually consists of 35–45 cycles. Depending on the number of copies of starting template and the type of instrument used for real-time PCR, up to 55 cycles may be performed. However, performing too many cycles will increase the background of nonspecific products and will lead to inaccurate melting curve analyses, since these are carried out after completing the run.

4. RT-PCR

When using the QuantiTect 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 QIAGEN Oligotex Kits for efficient isolation of mRNA and QIAGEN Omniscript™ or Sensiscript™ Reverse Transcriptases for reverse transcription (see page 37 for ordering information). Primers can be random hexamers, sequence-specific primers, or oligo-dT molecules. Use of oligo-dT primers results in reverse transcription of poly A^+ mRNA only.

5. Absolute and relative quantification

Target nucleic acids can be quantified using either absolute quantification or relative quantification. Absolute quantification determines the absolute amount of target (expressed as copy number or concentration), whereas relative quantification determines the ratio between the amount of target and a reference molecule, usually a suitable housekeeping gene. This normalized value can then be used to compare, for example, differential gene expression in different samples. An introduction to these methods is given below. For further details, refer to the literature provided with your real-time cycler.

5.1. Absolute quantification

The absolute amount of the target nucleic acid of interest is determined using external standards. These standards usually contain sequences that are the same as, or differ only slightly from the target sequence, and their primer binding sites are always identical to the target sequence. This ensures equivalent amplification efficiencies of standard and target molecules, which is essential for absolute quantification. A standard curve (plot of C_T values/crossing points of different standard dilutions against log of amount of standard) is generated using a dilution series of the standards. Amplification of the standard dilution series and of the target sequence is carried out in separate tubes. Comparing the C_T of unknown amounts of target with the standard curve allows calculation of the initial amount of target used in real-time PCR. It is important to select an appropriate standard for the type of nucleic acid to be quantified (see page 27).

5.2. Relative quantification

In this approach, the ratio between the amount of target molecule and a reference molecule within the same sample is calculated. This normalized value 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 molecule, 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 nucleic acid and the reference molecule are amplified with comparable or different efficiencies. For determination of PCR efficiency, see section 6.

- **Different amplification efficiencies**

  Amplification efficiencies of target and reference sequences are usually different, since primer binding sites, PCR product sequences, and PCR product sizes normally differ in each. If this is the case, we recommend performing two standard curves (see section 7), one for the target and one for the reference, using, for example, total RNA prepared from a reference cell line. Since the resulting standard curves will not be parallel, the differences in C_T values of the target and reference will not be constant when the amounts of target and reference are varied. The amounts of reference and target are calculated using the resulting C_T values of the sample and the corresponding standard curve. The ratio of the resulting amounts of target and reference in the sample of interest can then be determined. Given that the expression of the reference does not change in different samples, the ratio of the two PCR products varies according to the expression level of the target gene (e.g., in different tissues).

- **Comparable amplification efficiencies**

  If amplification efficiencies of target and reference sequence are the same, one standard curve is sufficient. The standard curve is performed as described in the previous section but with a dilution series of the reference only. Unknown amounts of target and reference in the sample are calculated by comparing theresulting C_T values with the standard curve of the reference sample.

  An alternative approach is the comparative method ($\Delta\Delta C_T$ method) which relies on comparing differences in C_T values. Preparation of standard curves is not necessary. This method can only be used if amplification efficiencies of target and reference sequence are nearly equivalent. For detailed information, refer to literature supplied with real-time instruments.

6. Determination of PCR efficiency

In order to compare the amplification efficiencies of two target sequences, prepare a dilution series for each target (targets A and B). Amplify each dilution series by PCR or two-step RT-PCR. Subtract the C_T values/crossing points of target A from the C_T values of target B. Plot the differences in C_T values/crossing points against the logarithm of the template amount. If the slope of the resulting straight line is <0.1, amplification efficiencies are comparable.

7. Generating standard curves

Standard curves can be used for both absolute and relative quantification. In order to generate a standard curve, at least 5 different concentrations of the standard should be measured, and the amount of unknown target should fall within the range tested. Reactions should be carried out in triplicate when using ABI Sequence Detection Systems or the iCycler, or in duplicate when using the LightCycler. When using very low amounts of template, reactions should be carried out in quadruplicate or triplicate, for ABI Sequence Detection Systems/iCycler and LightCycler, respectively.

8. Standards

For absolute quantification of DNA and RNA molecules (see page 25), 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

8.1 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 cDNA 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 determinations 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:

$$(X\ g/\mu l\ RNA\ /\ [transcript\ length\ in\ nucleotides \times 340]) \times 6.022 \times 10^{23} = Y\ molecules/\mu l$$

Example

Transcript length: 500 nucleotides

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

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

8.2. 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 belinearized 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 or cDNA.

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

$$(X\ g/\mu l\ DNA\ /\ [plasmid\ length\ in\ basepairs \times 660]) \times 6.022 \times 10^{23} = Y\ molecules/\mu l$$

Example

Plasmid length: 3000 bp

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

Calculation: $(100 \times 10^{-9}\ g/\mu l\ /\ [3000 \times 660]) \times 6.022 \times 10^{23} = 3.0 \times 10^{10}$ 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.

9. 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 8). 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.

10. Prevention of contamination using UNG

PCR products from previous PCR runs are a major source of PCR contamination, known as carryover contamination. This problem can be reduced by enzymatic destruction of contaminants.

To enable this step to be performed, dTTP in the QuantiTect SYBR Green PCR Master Mix is partially substituted by dUTP. In addition, the enzyme uracil-N-glycosylase (to be provided by the user) must be added to the PCR in an appropriate concentration (see protocol). An incubation step of 50°C for 2 minutes is added to the cycling program, before activation of HotStarTaq DNA Polymerase. During this incubation step, UNG removes uracil from dUMP incorporated into any contaminating molecules, leaving apyrimidinic sites. During the activation step of HotStarTaq DNA Polymerase (15 minutes at 95°C), the UNG is inactivated, and contaminating molecules are destroyed by cleavage at the abasic sites. During subsequent cycling, only target nucleic acid and not contaminating nucleic acid from previous reactions will be amplified.

If UNG treatment is carried out using heat-stable UNG from *E. coli*, all primers should have T_m >55°C, and PCR products should be refrigerated immediately after the run, because heat-stable UNG maintains a residual activity following the incubation step at 95°C for 15 minutes.

11. Data analysis

For data analysis, follow the recommendations provided by the manufacturer of the detection system. Data are produced as sigmoidal-shaped amplification plots (when using a linear scale), in which the fluorescence is plotted against the number of cycles. The threshold cycle (C_T) 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 increase in fluorescence. Determination of C_T values is carried out slightly differently on different kinds of cycler.

Typical Amplification Plot

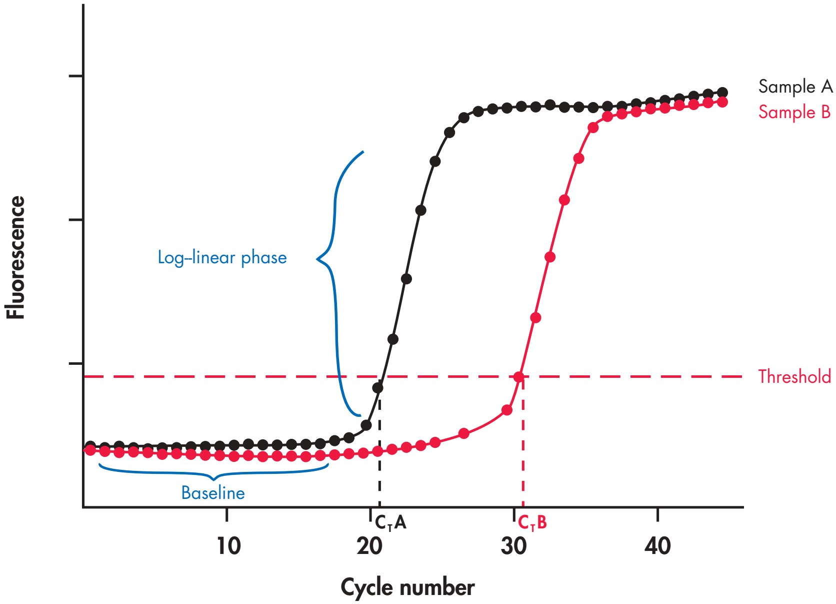

Figure 2. *Amplification plots showing increases in fluorescence from 2 samples (A and B). Sample A contains a higher amount of starting template than sample B.*

ABI detection systems

Baseline: The baseline is the noise level in early cycles, typically measured between cycles 3 and 15, where there is no detectable increase in fluorescence due to PCR products. The number of cycles used to calculate the baseline can be changed and should be reduced if high template amounts are used (see Troubleshooting). The baseline is subtracted from the fluorescence obtained from PCR products.

Threshold: The threshold is adjusted to a value above the baseline, but must be located in the log-linear range of the PCR and **never** in the plateau phase. When using the ABI PRISM 7700, begin by using the preset threshold, but check whether this is appropriate and adjust it if necessary. When using the ABI GeneAmp 5700, the user must select a suitable starting threshold and adjust this further if necessary. Setting of the threshold should be done using a logarithmic amplification plot so that the log-linear range of the curve can be identified easily.

Threshold cycle (C_T): This is the cycle at which the amplification plot crosses the threshold, i.e., at which there is the first clearly detectable increase in fluorescence.

LightCycler

There are two 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 ABI Sequence Detection Systems. Use the "arithmetic" mode of baseline adjustment when analyzing data obtained with SYBR Green.

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.

11. 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 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.

Melting Curve Analysis

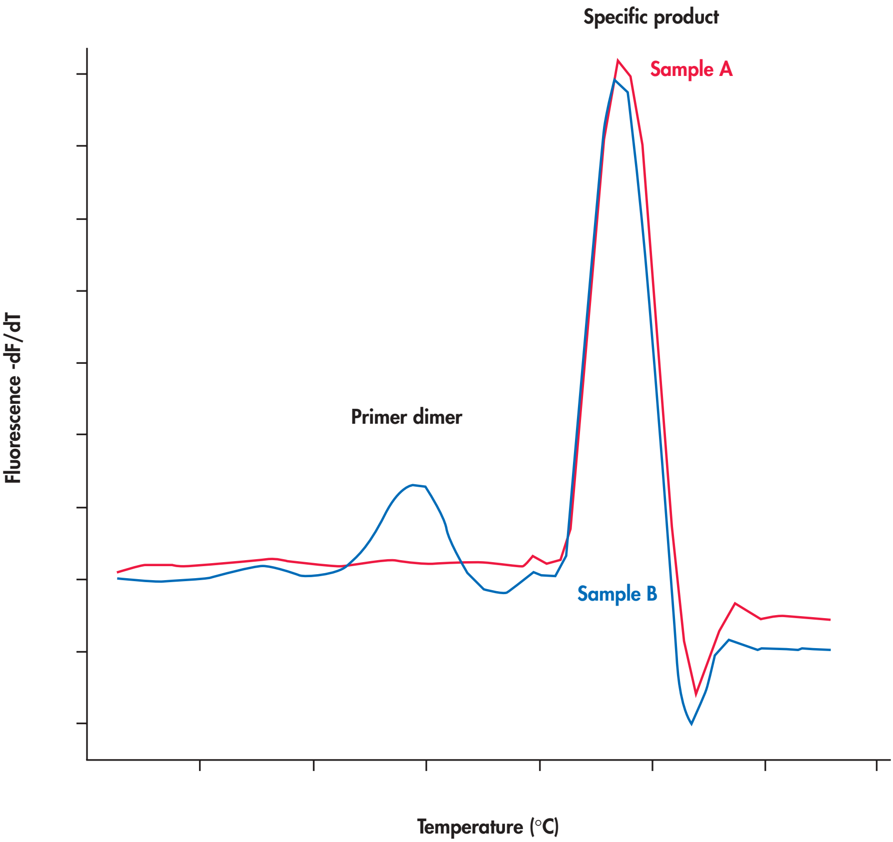

Figure 3. *Melting curve analysis of 2 samples (A and B). Sample A yields only 1 peak resulting from the specific amplification product (primer-dimers not co-amplified). Sample B shows a peak from the specific product and a peak at a lower temperature from amplification of primer-dimers.*

12. Optimization

If nonspecific products are amplified and/or sensitivity is low, several parameters can be optimized.

Detection temperature

Use melting curve analysis to determine the T_m of nonspecific products. In the next run, add a detection step after the elongation step to the cycling program. The temperature should be above the T_m of the nonspecific product but about 3°C below the T_m of the specific product (i.e., below the temperature where a specific peak becomes visible). The step should last from 5 to 15 seconds. Ensure that the instrument collects data only during this additional detection step. For further details, refer to the relevant protocol.

Primer concentration

Primer concentration can influence C_T but also affects the specificity of the reaction. Higher primer concentrations can lead to lowered C_T values but also increase the yield of nonspecific products such as primer-dimers. The primer concentrations recommended in the protocols normally work well, but if results are not satisfactory, primer titrations can be carried out (see protocols, pages 10 and 13 for further details).

It is sufficient to test equimolar amounts of each primer: carrying out a primer matrix (in which unequal amounts of each primer are tested) as recommended by some suppliers appears not to improve results further when using SYBR Green I for detection.

$MgCl_2$ concentration

When using the QuantiTect SYBR Green PCR Master Mix, it is usually not necessary to optimize the amount of $MgCl_2$. Adjusting the amount of $MgCl_2$ most often does not improve results and may even worsen them (especially when using the LightCycler). In exceptional cases, results may be improved by adjusting the concentration of $MgCl_2$, as determined by carrying out a titration of $MgCl_2$ levels (see protocols, pages 10 and 13 for further details).

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Ordering Information

Product	Contents	Cat. No.
QuantiTect SYBR Green PCR Kit — for quantitative real-time PCR and two-step RT-PCR		
QuantiTect SYBR Green PCR Kit	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect SYBR Green PCR Master Mix;* 2 x 2.0 ml RNase-free water	204143
Related products		
QuantiTect SYBR Green RT-PCR Kit — for quantitative real-time one-step RT-PCR		
QuantiTect SYBR Green RT-PCR Kit	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect SYBR Green PCR Master Mix;* 1 x 100 µl QuantiTect RT Mix, 2 x 2.0 ml RNase-free water	204243
QuantiTect Probe PCR and RT-PCR Kits — for quantitative, real-time PCR and RT-PCR using sequence-specific probes		
QuantiTect Probe PCR Kit (200)	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect Probe PCR Master Mix;† 2 x 2.0 ml RNase-free water	204343
QuantiTect Probe RT-PCR Kit (200)	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect Probe RT-PCR Master Mix;† 1 x 100 µl QuantiTect RT Mix; 2 x 2.0 ml RNase-free water	204443
QIAGEN Operon Oligonucleotide Synthesis Service — high-quality oligos, modified oligos, and longmers		
Oligonucleotide Synthesis Service	Custom-made oligonucleotides and a wide range of modified oligos, including Molecular Beacons, dual-labeled probes, and many more	Inquire

* *Contains 5 mM $MgCl_2$*

† *Contains 8 mM $MgCl_2$*

Ordering Information

Product	Contents	Cat. No.
Omniscript RT Kit — for reverse transcription using ≥50 ng RNA		
Omniscript RT Kit (10)*	For 10 reverse-transcription reactions: 40 units Omniscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix,[†] RNase-free water	205110
Sensiscript RT Kit — for reverse transcription using <50 ng RNA		
Sensiscript RT Kit (50)*	For 50 reverse-transcription reactions: Sensiscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix,[†] RNase-free water	205211
HotStarTaq DNA Polymerase — for highly specific hot-start PCR		
HotStarTaq DNA Polymerase (250 U)*	250 units HotStarTaq DNA Polymerase, 10x PCR Buffer,[‡] 5x Q-Solution, 25 mM $MgCl_2$	

* *Larger kit sizes available; please inquire*

† *Contains 5 mM each dNTP*

‡ *Contains 15 mM $MgCl_2$*

Ordering Information

Product	Contents	Cat. No.
QIAprep Kits — for rapid plasmid purification		
QIAprep 8 Miniprep Kit (10)*	For 10 x 8 high-purity plasmid minipreps: 10 QIAprep 8 Strips, Reagents, Buffers, Collection Microtubes (1.2 ml), Caps	27142
QIAamp Kits — for isolation of genomic and viral DNA from clinical sources		
QIAamp DNA Mini Kit (10)*	For 10 DNA preps: 10 QIAamp Spin Columns, Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51399
DNeasy Kits — for isolation of genomic DNA from a variety of sources		
DNeasy Tissue Kit (50)*	50 DNeasy Spin Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	69504
RNeasy Kits — for isolation of total RNA from a variety of sources		
RNeasy Mini Kit (20)*	20 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74103
Oligotex mRNA Kits — for isolation of poly A$^+$ mRNA from total RNA		
Oligotex mRNA Mini Kit (12)*†	For 12 mRNA minipreps: 200 µl Oligotex Suspension, Small Spin Columns, Collection Tubes (1.5 ml), RNase-free Reagents and Buffers	70022

* *Larger kit sizes available; please inquire*

† *Not available in Japan*

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