iQ™ SYBR® Green Supermix

Instruction Manual

Catalog # 170-8880
170-8882
170-8884
170-8885

BIO-RAD

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1. General Information

Shipping, Storage, and Stability

Shipping iQ™ SYBR® Green supermix is shipped on dry ice.

Storage and Stability iQ™ SYBR® Green supermix is stable for 12 months when stored in a constant temperature freezer at –20°C (frost-free freezers are not recommended), protected from light. For convenience, it may be stored at 4°C for up to 6 months. Repeated freezing and thawing of the supermix is not recommended.

Components of iQ™ SYBR® Green Supermix

2x iQ™ SYBR® Green supermix contains SYBR® Green I dye, 50 U/ml iTaq™ DNA polymerase, dNTPs (0.4 mM each of dATP, dCTP, dGTP, and dTTP), 6 mM $MgCl_2$, 40 mM Tris-HCl, pH 8.4, 100 mM KCl, 20 nM fluorescein, and stabilizers

Kit Contents

Catalog #	# of Reactions	Volume
170-8880	100 x 50 µl	1.25 ml x 2 vials
170-8882	500 x 50 µl	1.25 ml x 10 vials
170-8884	1,000 x 50 µl	1.25 ml x 20 vials
170-8885	2,000 x 50 µl	50 ml (1 bottle)

Quality Control

Each batch of iQ™ SYBR® Green supermix undergoes stringent functional testing to ensure sensitivity, specificity, linearity, and lot-to-lot consistency. Every batch is free of contaminating nucleases. Functionally, the supermix is tested to demonstrate resolution over several orders of dynamic range of cDNA and human genomic DNA.

Warranty

When stored at –20ºC (protected from light), Bio-Rad warrantees the iQ™ SYBR® Green supermix until the expiration date listed on the label.

Certificate of Analysis

To obtain a certificate of analysis that describes the quality control data for a specific lot, please contact your local Bio-Rad Laboratories office on the web at **bio-rad.com**. In the U.S., email the request for a certificate to **lsg.techserv.us@bio-rad.com**, or call Technical Support at 800-424-6723. When making the request, be sure to include the lot number and catalog number listed on the iQ™ SYBR® Green supermix label.

MSDS

To obtain an MSDS, go to **bio-rad.com**, and enter the catalog number of the product into the Search box. Select **MSDS** from the pull-down menu and click **Search**. If the MSDS is not available on the web site, please call your local Bio-Rad office.

2. Introduction

Product Information

Real-time quantitative PCR (qPCR) is a powerful advancement of the basic PCR technique. Through the use of appropriate fluorescent detection strategies, proper instrumentation, and optimized reagents, the starting amount of nucleic acid in the reaction can be measured accurately during the amplification process without the need for post-PCR gel analysis.

iQ™ SYBR® Green supermix is a convenient, ready-to-use, 2x reaction cocktail that is formulated for optimal results in qPCR assays based on SYBR® Green I detection. The supermix is designed to maximize the reproducibility, specificity, and efficiency of qPCR experimental results. The preblended mix contains an optimized concentration of SYBR® Green I dye, iTaq™ DNA polymerase, dNTPs, and buffers for detecting a wide range of targets. The formulation supports short denaturation, annealing, and extension times for faster reactions, and promotes stringent primer binding during the annealing step. The supermix also contains fluorescein for well-factor collection on the MyiQ™, iCycler iQ®, and iQ™5 real-time PCR detection systems.

SYBR® Green I Dye

SYBR® Green I dye is the most commonly used double-stranded (dsDNA)-binding dye for real-time PCR. The dye exhibits minimal fluorescence when free in solution, but when bound to dsDNA, its fluorescence increases up to 1,000-fold. In PCR, its fluorescent signal increases in proportion to the amount of dsDNA generated during amplification (Figure 1). The excitation and emission maxima of SYBR® Green I dye are at 494 nm and 521 nm, respectively, making the dye compatible for use with any real-time cycler.

The presence of a dsDNA-binding dye in the iQ™ SYBR® Green supermix allows for simplified assay design without the need for additional fluorescent probes, and enables assay verification using a melt-curve analysis.

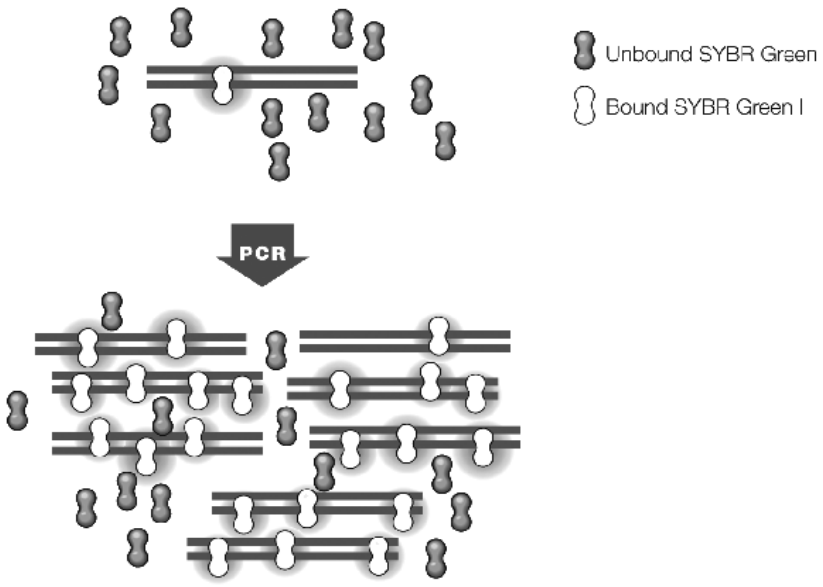

Figure 1. SYBR® Green I DNA-binding in a real-time PCR reaction.

iTaq DNA Polymerase

iQ™ SYBR® Green supermix is preblended with iTaq DNA polymerase for optimal qPCR results. iTaq DNA polymerase is an antibody-mediated hot-start polymerase suitable for both conventional and real-time PCR applications. The antibody sequesters polymerase activity prior to the initial PCR denaturation step at 95°C. Upon a quick, 3-minute heat activation, the antibody denatures irreversibly, releasing the fully active enzyme.

iTaq DNA polymerase is highly specific and sensitive, and provides improved product yield over a broad range of targets compared with standard chemically-modified hot-start DNA polymerases.

Fluorescein Passive Reference Dye

iQ™ SYBR® Green supermix contains fluorescein for use as a passive reference dye. The addition of fluorescein provides sufficient background fluorescence prior to amplification, enabling well factor collection from the same plate that contains test samples. Fluorescein does not interfere with PCR efficiency or sensitivity. It is used to collect dynamic well factors on MyiQ, iCycler iQ, and iQ5 real-time PCR detection systems.

3. Guidelines for Optimizing qPCR Reactions

Overview

Specificity and efficiency are two essential requirements for successful real-time PCR amplification. Poor assay design can result in problems, such as nonspecific product formation and inefficient primer association with the target sequence, which can dramatically affect data quality. Nonspecific amplification and primer-dimer formation can greatly reduce amplification efficiency of the target gene by competing for reaction components.

Well-designed real-time PCR reactions enable accurate quantification of nucleic acid in a sample. Proper assay design and optimization ensure that the threshold cycle (C_T) value, which is recorded during the reaction, accurately reflects the starting amount of template.

Both the primers and target sequence can affect the efficiency and specificity amplification. Therefore, care must be taken when choosing a target sequence and designing primers. In addition, the optimized assay must be validated for sensitivity and specificity to determine robustness and amplification efficiency over a broad dynamic range.

Target Identification

When choosing a region of the target to amplify, follow these guidelines:

- Amplify a 75–200 bp product. Short products are typically amplified with higher efficiency than longer ones; however, a product should be at least 75 bp to be easily distinguished from primer-dimers that could potentially form
- Avoid regions that have secondary structure. Use programs, such as mfold (**http://www.bioinfo.rpi.edu/applications/mfold/**), for nucleic acid folding and hybridization prediction. See Bio-Rad bulletin 2593, *Real-Time PCR: General Considerations*, for more details
- Avoid regions with long (>4) repeats of single bases
- Choose a region that has a GC content of 50–60%

Designing Primers

When designing new primers, follow these guidelines:

- Design primers that have a GC content of 50–60%
- Strive for a melting temperature (T_m) between 50 and 65°C. One way to calculate T_m values is to use the nearest neighbor method. Primer 3 is a widely used program for designing primers (**http://frodo.wi.mit.edu/primer3/input.htm**). Concentration values of 50 mM for salt and 300 nM for oligonucleotide are required for generating primers using Primer 3
- Avoid secondary structure; adjust primer locations so that they are outside regions of secondary structure in the target sequence, if needed
- Avoid repeats of Gs or Cs longer than 3 bases, and place Gs and Cs on ends of primers
- Check the sequence of forward and reverse primers to ensure no 3' complementarity (avoid primer-dimer formation)
- Verify specificity using tools such as the Basic Local Alignment Search Tool (BLAST) (**http://www.ncbi.nlm.nih.gov/blast/**)
- Consider using either a minor groove binder (MGB) or a few locked nucleic acid (LNA) moieties when working on sequences with unusually low GC content or if the primer location cannot be moved to a more GC rich region (Ugozzoli et al. 2004, Latorra et al. 2003)

For a list of online tools to help you with primer design, go to **www.bio-rad.com/genomics/pcrsupport/** and click on **Assay Design**. Commercially available programs, such as Beacon Designer Software, perform both primer design and target sequence selection.

Optimizing the Annealing Temperature

Optimizing the annealing temperature of a real-time PCR assay is one of the most critical parameters for ensuring reaction specificity. Setting the annealing temperature too low may lead to amplification of nonspecific PCR products. Alternatively, setting the annealing temperature too high may reduce the yield of a desired PCR product.

Even after calculating the T_m of a primer, you may need to determine the annealing temperature empirically. This process involves repeating a reaction at many different temperatures, which is easy when using qPCR instruments that have a thermal gradient feature. Bio-Rad's real-time systems offer a gradient feature, which enables a range of temperatures to be tested simultaneously, so the annealing temperature can be optimized in a single experiment.

To find the optimal annealing temperature for the reaction, test a range of temperatures above and below the calculated T_m of the primers. The optimal annealing temperature is the one that results in the lowest C_T with no nonspecific amplification. Verify that there is no nonspecific amplification with a melt curve and gel analysis (see “Assay Specificity Verification with a Melt Curve Analysis” on page 8). PCR sample gel analysis can be a potential source of contamination for real-time PCR and must be handled with care. Dedicated gel analysis area and pipets are strongly recommended.

Assay Validation

A common method for validating a real-time PCR assay involves constructing a standard curve. The standard curve helps you to determine the efficiency, linear dynamic range, and reproducibility of the assay. The efficiency of the assay (E) should be 90–105%, the R^2 of the standard curve should be >0.980 or r > |–0.990|, and the C_T values of the replicates should be similar. For more information about determining the efficiency of a reaction, see the *Real-Time PCR Applications Guide* (catalog #170-9799).

To demonstrate that results from your samples are within the dynamic range of the assay, the range of template concentrations used for the standard curve must encompass the entire range of template concentrations of your samples. If your samples give results outside the range of the standard curve, perform one of the following steps:

- Construct a wider standard curve covering your sample concentrations and perform an analysis to ensure that the assay is linear in that new range
- If the C_T of your samples is lower than that of the highest standard concentration used, repeat the assay and dilute your samples
- If the C_T of the your samples is higher than that of the lowest standard concentration used, repeat the assay using larger amounts of your samples

If your real-time PCR assay fails validation, you may need to redesign your primers and target. (See “Assay Specificity Verification with a Melt Curve Analysis” on page 8 for more details.)

4. Protocols

Compatible Instruments

iQ™ SYBR® Green supermix is validated for use on all Bio-Rad real-time PCR systems. When using a non-Bio-Rad real-time PCR system that requires ROX for normalization of fluorescent signals, iQ™ SYBR® Green supermix should be supplemented with additional ROX passive reference dye (catalog #172-5858). Alternatively, iTaq™ SYBR® Green supermix with ROX (catalog #172-5851) or iTaq™ fast SYBR® Green supermix with ROX (catalog #172-5101) can be used. Contact the real-time instrument manufacturer for information on the appropriate ROX concentration that should be used for your specific instrument.

Additional Materials Required

In addition to the iQ™ SYBR® Green supermix, the following materials are required to set up a qPCR reaction:

- Template (cDNA, genomic DNA, or plasmid DNA)
- Forward and reverse primers
- Pipets and pipet tips (aerosol barrier tips recommended)
- Sterile microcentrifuge tubes (screwcaps recommended)
- Microcentrifuge
- Vortex mixer
- Optical qPCR microplates or tubes
- Nuclease-free water
- Gloves

NOTE: For a complete list of Bio-Rad products available for use with iQ™ SYBR® Green supermix, see page 13.

Reaction Set Up

Use the following protocol to set up and run a PCR reaction using the iQ™ SYBR® Green supermix.

1. Thaw the frozen 2x iQ™ SYBR® Green supermix, template, and primers on ice. Gently mix each tube to ensure thorough resupension of components before use. Briefly spin the tubes in a microcentrifuge to collect contents at the bottom of the tubes.

2. Add the components into each reaction tube in the order shown in Table 1. Briefly, vortex each reaction mix, and spin the tubes at low speed in a microcentrifuge.

Table 1. Reaction mix components required to set up a 20 or 50 µl reaction.

Step	Volume Per Reaction		Final Concentration
Nuclease-free water	Variable	Variable	–
Forward primer	Variable	Variable	300 nM (100–500 nM)
Reverse primer	Variable	Variable	300 nM (100–500 nM)
iQ™ SYBR® Green supermix (2x concentration)	10 µl	25 µl	1x
Template (genomic DNA or cDNA)	Variable	Variable	• An amount of cDNA equivalent to 1 pg to 100 ng of total RNA • 5 pg to 50 ng of genomic DNA
Total reaction volume	**20 µl**	**50 µl**	

TIP: Prepare 10% more reaction mix than required to compensate for potential pipetting loss. Assemble replicate samples as a master mix with a single addition of the sample template.

TIP: Include no template control and positive control reactions for detecting and quantifying possible contamination.

3. Load reaction replicates into PCR tubes or microplates, and seal the reaction vessels. For best results, briefly spin the microplate or tubes in a microcentrifuge at low speed to remove any bubbles. If necessary, store the sealed reactions on ice or at 4°C until you are ready to start running the real-time PCR protocol.

4. Program the thermal cycler with the recommended real-time PCR protocol with or without a melt curve step (Table 2 and Table 3).

   NOTE: When using the MyiQ™, iCycler iQ®, and iQ™5 real-time PCR systems, always collect dynamic well-factors to compensate for system or pipetting variation.

5. Place the sealed reaction vessels in the thermal cycler block, and start running the PCR protocol.

   NOTE: PCR products can be stored at –20°C after the run.

Optimized Cycling Protocols

The recommended 2- or 3-step optimized cycling conditions with or without a melt curve step are shown in Table 2 and Table 3, respectively. Program the thermal cycler with one of these real-time PCR protocols. The cycling protocol may need to be modified for fast thermal cyclers.

Table 2. A typical 2-step real-time PCR protocol.

Cycling Step	Temperature	Hold Time (min:sec)	# of Cycles
Initial denaturation and enzyme activation	95°C	2:00–3:00	1
Denaturing	95°C	0:10–0:15	40
Annealing and extension	55–60°C	0:30	
(***Optional***) Melt curve	55–95°C (in 0.5°C increments)	0:10–0:30	1

Table 3. A typical 3-step real-time PCR protocol.

Cycling Step	Temperature	Hold Time (min:sec)	# of Cycles
Initial denaturation and enzyme activation	95°C	2:00–3:00	1
Denaturing	95°C	0:10–0:15	40
Annealing	55–60°C	0:15–0:30	
Extension	72°C	0:30	
(***Optional***) Melt curve	55–95°C (in 0.5°C increments)	0:10–0:30	1

5. Data Analysis

qPCR Analysis Methods

When performing qPCR, it is necessary to choose the appropriate reaction conditions that will generate robust and efficient amplification for both relative and absolute quantitation. Absolute and relative quantification use the C_T values obtained during qPCR:

- In absolute quantification, the C_T values of test samples are compared to those of standards of known quantity using a standard curve. This analysis yields the copy number or concentration of a sample, and is commonly used for pathogen detection or copy number analysis
- In relative quantification, the C_T values of test samples are compared to those of control samples. This analysis yields the ratio of the relative amount (fold difference) of a target nucleic acid in test vs. control samples, and is commonly used for gene expression studies

For both absolute and relative quantification, the amount of sample is defined by comparing to a set of standards. In absolute quantification, known quantities of standards are used to normalize the calculated target quantity to a unit amount of sample. In relative quantification, standards are used to ensure that the target quantities are compared from equivalent amounts of samples. Although the standard for either quantification method can be the number of cells used for template preparation, nanograms of nucleic acid used as PCR template, or the expression level of a reference gene, the first two are more commonly used for absolute quantification, whereas the third is typically used for relative quantification.

For additional information on various qPCR analysis methods, refer to the citations on page 9.

Assay Specificity Verification with a Melt Curve Analysis

Melt-curve analysis can be used to identify the reaction products, including nonspecific products and primer-dimers, when fluorescence of the assay's reporter chemistry depends on the presence of dsDNA (as with SYBR® Green I dye). This is valuable because the presence of nonspecific products and primer-dimers can severely reduce the amplification efficiency and ultimately the accuracy of the data. Primer-dimers can also limit the dynamic range of the desired standard curve due to competition for reaction components during amplification.

After completion of the amplification reaction, a melt curve is generated by increasing the temperature in small increments and monitoring the fluorescent signal at each step. As the dsDNA in the reaction denatures or "melts", the fluorescence decreases rapidly and significantly. A plot of the negative first derivative of the change in fluorescence plotted as a function of temperature (–dF/dT) has distinct peaks that correspond to the T_m of each product (Figure 2A). The T_m of each product is defined as the temperature at which the corresponding peak maximum occurs. This analysis can confirm the specificity of the chosen primers, as well as reveal the presence of primer-dimers. Gel analysis can also be used to verify the absence of nonspecific amplification (Figure 2B).

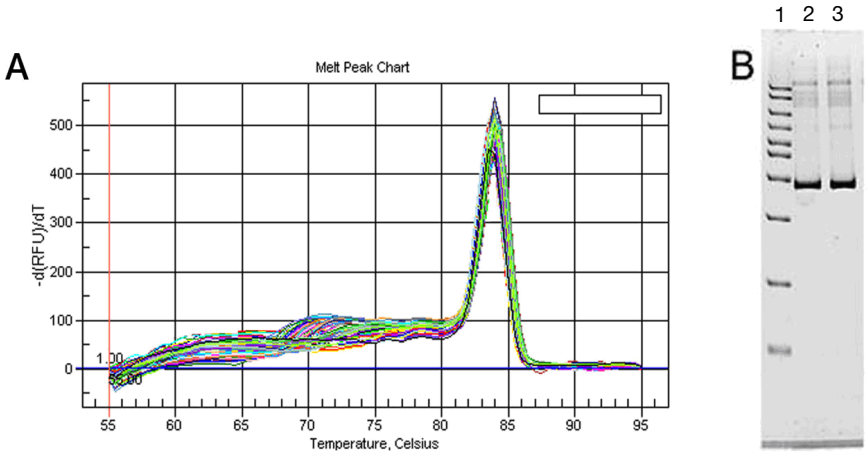

Figure 2. Assay specificity analysis of products from a SYBR® Green I assay. A, a melt curve. A single melt curve peak indicates the reaction is specific for one PCR product. **B**, a gel analysis of the PCR product. Lane 1, AmpliSize® DNA ladder (50–20,000 bp); lanes 2 and 3, two replicates of the PCR product from the reaction shown in **A**.

A melt peak can confirm the specificity of the chosen primers and distinguish the desired product from primer-dimers and nonspecific products. Because of their small size, primer-dimers usually melt at lower temperatures than the desired product, whereas nonspecific amplification can result in PCR products that melt at temperatures above or below that of the desired product.

TIP: The melt-curve peak can also be used to determine the optimal annealing temperature for the reaction. (See “Optimizing the Annealing Temperature” on page 4 for more details.)

Well Factor Collection

Well factors are used to compensate for any optical system or pipetting non-uniformity, and are used to optimize fluorescent data quality and analysis. iQ™ SYBR® Green supermix contains a small amount of fluorescein that permits the collection of dynamic well factors on MyiQ™, iCycler iQ®, and iQ™5 real-time systems, without interfering with the PCR.

Well factors are collected either directly from an experimental plate or indirectly from an external source plate. The more accurate source of well factors is the actual experimental plate. If every well contains the same fluorophore and sample volume, experimental well factors, also called dynamic well factors, should be used. If wells contain different fluorophores or sample volumes, an external well factor plate must be used. In either case, well factors must be collected at the beginning of each experiment, before the initial 95°C step in the protocol.

Please refer to the real-time instrument manual for the well factor collection method recommended by the instrument manufacturer.

6. Useful References

References for Data Analysis

Bustin SA (2000). Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. J Mol Endorinol 25, 169–193.

Bustin SA (2002). Quantification of mRNA using real-time reverse transcription PCR (RT-PCR): trends and problems. J Mol Endocrinol 29, 23–29.

Bustin SA (2002). Real-Time PCR. In Encyclopedia of Diagnostic Genomics and Proteomics, Fuchs J. and Podda M., ed. (Marcel Dekker Inc. New York), pp. 1117–1125.

Bustin, SA (2004). A–Z of Quantitative PCR. (California: IUL Biotechnology Series, International University Line).

Bustin SA et al. (2009). The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem 55, 611–622.

Hellemans J et al. (2007). qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. Genome Biol 8, R19.

Livak KJ et al. (1995). Towards fully automated genome-wide polymorphism screening. Nat Genet 9, 341–342.

Livak KJ and Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25, 402–408.

Pfaffl MW (2001). A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29, 2002–2007.

Vandesompele J et al. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 3, 1–12.

References for Designing Primers

Latorra D et al. (2003). Design considerations and effects of LNA in PCR primers. Mol Cell Probes 17, 253–259.

Pattyn F et al. (2003). RTPrimerDB: the Real-Time PCR primer and probe database. Nucleic Acids Res 31, 122–123.

Ugozzoli LA et al. (2004). Real-time genotyping with oligonucleotide probes containing locked nucleic acids. Anal Biochem 324,143–152.

Wu DY et al. (1991). The effect of temperature and oligonucleotide primer length on the specificity and efficiency of amplification by the polymerase chain reaction. DNA and Cell Biol, 10, 233–238.

7. Troubleshooting

An optimal qPCR reaction results in successful amplification over a broad dynamic range. Consult the following tables for troubleshooting tips and solutions.

Poor Signal or No Yield

Possible Cause	Solution(s)
Degraded template material Degradation can occur during sample preparation or template storage. Long-term storage of nucleic acid templates in dilute solutions can contribute to template degradation	• Do not store diluted template in water or at low concentrations • Check the integrity of template material by automated or manual gel electrophoresis • Use freshly prepared nucleic acid to ensure the availability of intact template sequence for efficient amplification • Use PCR tubes, plates and pipet tips with low DNA retention
Inadequate thermal cycling conditions Insufficient incubation times do not allow complete replication of template, and can result in a lack of amplification signal	• Appropriate denaturation and extension times must be used depending on the template used. • Generally large amplicons in genomic DNA templates may need longer denaturation and extension times than cDNA and plasmid templates. Also use instrument-specific guidelines while optimizing denaturation and extension times
PCR inhibitor present Sometimes inhibitors of PCR are carried over from sample preparation (nucleic acid extraction). Common inhibitors include phenol, detergents, proteases, and residual compounds from source materials, such as animal or plant tissue, bodily fluids, or soil preparations	• Perform a dilution series of the PCR template to determine whether the effect of the inhibitory agent can be reduced • Take extra care during the nucleic acid extraction steps to minimize carryover of PCR inhibitors

Signal in Negative Control

Possible Cause	Solution(s)
Contamination of reaction components with target sequence Poor laboratory techniques can result in contamination of PCR samples. Contamination is evidenced by the presence of an amplification signal in negative control samples prepared with all reaction components except the DNA template, referred to as a "no-template" or "primer-only" control	• Designate a work area exclusively for PCR assay setup • Use a solution of 10% bleach instead of ethanol to prepare the work area for PCR assay setup. Ethanol only induces precipitation of DNA in your work area, while the 10% bleach solution hydrolyzes, as well as dissolves, any residual DNA • Post-PCR gel analysis samples can be a potential source of contamination and must be handled carefully. Dedicated gel-analysis area and pipets are recommended to avoid contamination with non-specific PCR products In general, follow these practices to minimize the risk of sample contamination: • Wear gloves • Use sterile screwcap tubes • Use aerosol barrier pipet tips • Use calibrated pipets dedicated to PCR • Use nuclease-free water and use it only for PCR • Use a no-template control to verify the absence of contamination • Prepare a reaction mix with sufficient volume (10% more than required) to prepare all replicate samples
Primer-dimer formation Primer-dimers may be the result of poor primer design or high primer concentrations	• Reduce the primer concentration • Evaluate primer sequences for complementarity and secondary structure. Redesign primers if necessary • Use gradient feature and perform melt-curve analysis to determine if primer dimers are present • Perform a melt-curve analysis to determine if primer-dimers are present
Amplification of genomic DNA In RT-qPCR reactions, the RNA used for reverse transcription (RT) must be free of genomic DNA contamination. If the PCR primers for the cDNA template are able to anneal to the genomic copy of the same gene, an amplification signal might be observed in the no-RT controls. If SYBR® Green I dye is used, a melt-curve analysis is likely to reveal a peak T_m in the no-RT controls that may or may not be similar to that of the expected cDNA amplicon	• Treat samples with purified RNase-free DNase before reverse transcription • Design primers at splice junctions to avoid genomic DNA amplification

C_T Values Differing Across Replicate Samples

Possible Cause	Solution(s)
Pipetting errors C_T values of replicates can show increased variation due to poor laboratory technique or imprecise pipets	In general, follow these practices to improve reproducibility of replicate samples: • Wear gloves • Use sterile screwcap tubes • Use aerosol barrier pipet tips • Use calibrated pipets dedicated to PCR • Use nuclease-free water • Use a no template control to verify the absence of contamination • Prepare a reaction mix in a separate tube for all replicate samples • Add template to reaction mix before pipetting into reaction vessels • Avoid pipetting volumes <5 µl
Primer Design Some primers are particularly sensitive to thermal cycling conditions, leading to poor reproducibility in amplification reactions	Verify how your primers behave at different annealing temperatures by using a temperature gradient (page 8)

8. Ordering Information

Bio-Rad Laboratories provides a wide selection of products for running real-time PCR reactions. For a complete list of products, consult the Gene Expression Gateway at **www.bio-rad.com/genomics/** and the Bio-Rad Life Science Research web site at **www.bio-rad.com/amplification**.

Reagents for Real-Time PCR

Catalog Number	Description
Supermixes for qPCR	
170-8880	iQ™ SYBR® Green supermix, 100 x 50 µl reactions
170-8882	iQ™ SYBR® Green supermix, 500 x 50 µl reactions
170-8884	iQ™ SYBR® Green supermix, 1,000 x 50 µl reactions
170-8885	iQ™ SYBR® Green supermix, 2,000 x 50 µl reactions
172-5849	iQ multiplex powermix, 200 x 50 µl reactions
172-5201	SsoFast™ EvaGreen® supermix, 500 x 20 µl reactions
172-5106	iTaq™ fast supermix with ROX, 500 x 20 µl reactions
172-5855	iTaq supermix with ROX, 500 x 50 µl reactions
172-5851	iTaq™ SYBR® Green supermix with ROX, 500 x 50 µl reactions
172-5101	iTaq™ fast SYBR® Green supermix with ROX, 500 x 20 µl reactions
170-8861	iQ supermix, 500 x 50 µl reactions
Reverse Transcription and RT-qPCR Reagents	
170-8891	iScript™ cDNA synthesis kit, 100 x 20 µl reactions
170-8897	iScript Select cDNA synthesis kit, 100 x 20 µl reactions
170-8893	iScript one-step RT-PCR kit with SYBR® Green, 200 x 50 µl reactions
170-8895	iScript one-step RT-PCR kit for probes, 200 x 50 µl reactions
Additional Consumables	
170-8872	$MgCl_2$ solution, 50 mM, 1.25 ml
172-5858	ROX passive reference dye, 0.5 ml

Microplates, Tubes, and Sealing Options

Catalog Number	Description
Tubes	
TLS-0801*	Low-profile 0.2 ml 8-tube strips without caps, natural, 120 strips (960 tubes)
TLS-0851*	Low-profile 0.2 ml 8-tube strips without caps, white, 120 strips (960 tubes)
TBS-0201*	Full-height 0.2 ml 8-tube strips without caps, natural, 125 strips (1,000 tubes)
48-Well Plates	
MLL-4801*	Multiplate™ low-profile 48-well unskirted PCR plates, natural, 50

Microplates, Tubes, and Sealing Options (continued)

Catalog Number	Description
MLL-4851*	Multiplate low-profile 48-well unskirted PCR plates, white, 50
MLP-4801*	Multiplate full-height 48-well unskirted PCR plates, natural, 50
96-Well Plates	
HSP-9601*	Hard-Shell® low-profile 96-well skirted PCR plates, white shell, clear well, 50
HSP-9655*	Hard-Shell low-profile 96-well skirted PCR plates, white shell, white well, 50
HSP-9901*	Hard-Shell low-profile 96-well skirted PCR plates, white shell, clear well, bar coded, 50
HSP-9955*	Hard-Shell low-profile 96-well skirted PCR plates, white shell, white well, bar coded, 50
HSS-9601*	Hard-Shell full-height 96-well semi-skirted PCR plates, clear shell, clear well, 25
HSS-9665*	Hard-Shell full-height 96-well semi-skirted PCR plates, black shell, white well, 25
MLL-9601*	Multiplate low-profile 96-well unskirted PCR plates, natural, 25
MLL-9651*	Multiplate low-profile 96-well unskirted PCR plates, white, 25
MLP-9601*	Multiplate full-height 96-well unskirted PCR plates, natural, 25
223-9441	iQ 96-well semi-skirted PCR plates, 25
384-Well Plates	
HSP-3801*	Hard-Shell 384-well skirted PCR plates, clear shell, clear well, 50
HSP-3805*	Hard-Shell 384-well skirted PCR plates, clear shell, white well, 50
Sealers	
TCS-0803*	Optical flat 8-cap strips, for 0.2 ml tubes and plates, ultraclear, 120 strips
MSB-1001*	Microseal® 'B' adhesive seals, optically clear, 100

*Other color options or packaging sizes available. Check Bio-Rad catalogs for a complete list of color, packaging, and barcode options.

Real-Time PCR Systems

Catalog Number	Description
Real-Time PCR Detection Modules	
185-5096	CFX96™ real-time PCR detection system, includes C1000™ thermal cycler chassis, CFX96 optical reaction module, CFX Manager™ software, cables, reagent and consumables
170-9770	MyiQ™ single-color real-time PCR detection system, includes iCycler® chassis, MyiQ optics module, 96-well reaction module, iQ5 optical system software, version 2.0, iQ supermix and iQ™ SYBR® Green supermix, accessories
170-9780	iQ™5 real-time PCR detection system, includes iCycler chassis, iQ5 optics module, 96-well reaction module, iQ5 optical system software, version 2.0, iQ supermix and iQ™ SYBR® Green supermix, accessories

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