GenePharma

SYBR Real-Time PCR Kit

For Amplification and detection of DNA in

Quantitative real-time PCR (qPCR).

Catalog No. QPG-040/QPG-041/QPG-042/QPG-043

User Manual

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Kit Contents and Storage

Cat. No. QPG-040	**Size: 100 rxns**
Cat. No. QPG-041	**Size: 200 rxns**
Cat. No. QPG-042	**Size: 500 rxns**

SYBR Real-Time PCR Kit Reagents

SYBR Real-Time PCR Kit Reagents box includes the following items.
Store components at –20℃. **Reagents must be stored in the dark.**

Reagent	Amount			Storage
	100 rxns	200rxns	500 rxns	
SYBR PCR Master Mix（2×Conc.）	2×1.0 ml	4×1.0 ml	5×2.0 ml	4℃
Taq DNA polymerase（5U/μl）	40 μl	80 μl	200 μl	-20℃
Sterilized ddH_2O	1.0 ml	2×1.0 ml	2×1.0 ml	4℃

Accessory Products

Accessory Products

Some of the reagents supplied in the SYBR Real-Time PCR Kit as well as other products suitable for use with the kit are available separately from Genepharma. Ordering information is provided below. For more information, refer to our Web site (**www.genepharma.com**).

Item	Amount	Catalog no.
RT-PCR Kit	20 rxns	QPG-090
	50 rxns	QPG-091
One-Step RT-PCR Kit	25 μl×25 rxns	QPG-060
	25 μl×50 rxns	QPG-061
Real-time PCR Core Reagent	100 rxns	QPG-070
	200 rxns	QPG-071
	300 rxns	QPG-072
One-Step SYBR Real-Time RT-PCR Kit	25μl×25 rxns	QPG-050
	25μl×50 rxns	QPG-051
	25μl×100 rxns	QPG-052

Introduction

Overview

Introduction

The SYBR® Real-Time PCR Kit provides qualified reagents in lyophilized form for the amplification and detection of DNA in quantitative real-time polymerase chain reaction (qPCR). The SuperMix formulation is aliquoted into plate wells or strip wells and then lyophilized for room temperature storage and ease of reaction setup. To perform PCR, simply add water, primers, and template, vortex to dissolve the pellet, and proceed with the reaction.

The system enables highly sensitive detection from as few as 10 copies of a target gene, with a broad dynamic range that supports accurate quantification of high-copy gene from 1 pg up to 1 μg of DNA:

- **2×SYBR® Green Reaction Mix** consists of a proprietary buffer system, SYBR® Green I, $MgSO_4$, dNTPs, and stabilizers. SYBR® Green I is a fluorescent dye that binds directly to double-stranded DNA (dsDNA). In qPCR, as dsDNA accumulates, the dye generates a signal that is proportional to the DNA concentration and that can be detected using real-time qPCR instruments. SYBR® Green I in this ready to use formulation can quantify as few as 10 copies of a target gene in as little as 1 pg of total DNA or RNA. It has a broad dynamic range of seven orders of magnitude, and is compatible with melting curve analysis.

Uses for the SYBR® Real-Time PCR Kit

Use the One-Step SYBR® Real-Time RT-PCR Kit in your experiments for the following purposes:

- To quantify a target gene in DNA, especially the low expression genes.
- To analyze the relative expression ratio between a target gene and a housekeeping gene.

Components of the Kit

Introduction

This section provides more information about the reagents supplied in the One-Step SYBR® Real-Time RT-PCR Kit.

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SYBR PCR Master Mix 2×Conc.	The 2×SYBR® Green Reaction Mix consists of a proprietary buffer system, SYBR® Green I, $MgCl_2$, dNTPs, and stabilizers. The mix includes 0.4 mM of each dNTP and 6mM $MgCl_2$, and has been confirmed to be work well for most targets in restrict lab experiments. However, the optimal Magnesium concentration may range from 3 to 6 mM, and so if necessary, use the separate tube of 25mM magnesium sulfate to increase the magnesium concentration.
	It's very important for you to store the SYBR PCR Master Mix in the dark.
Taq DNA polymerase 5U/μl	For most PCR reaction, the final concentration of DNA polymerase was ussually 0.05U/μl per reaction volume.

Method

Performing Real-Time PCR

Introduction

In SYBR® Green real-time PCR, as dsDNA accumulates, the dye generates a signal that is proportional to the DNA concentration and that can be detected using real-time qPCR instruments. This section provides guidelines and an example protocol for performing SYBR real-time PCR.

Instrument Compatibility

This kit can be used with a variety of real-time instruments, including but not limited to the ABI PRISM 7000, 7700, and 7900HT; the ABI 7300 and 7500 Real-Time PCR Systems; the Bio-Rad iCycler; the Stratagene Mx3000P and Mx4000; the Corbett Research Rotor-Gene 3000; the MJ Research DNA Engine Opticon, Opticon 2, and Chromo 4 Real-Time Detector; and the Cepheid Smart Cycler Optimal cycling conditions will vary slightly with different instruments.

Primer design & Concentration

Primer selection is one of the most important parameters for qPCR when using a SYBR Green detection system. When designing primers, keep in mind that the amplicon length should be approximately 80 – 250 bp. We strongly recommend using a primer design program such as Oligo 6.0.

Ensure that primers are specific for the target sequence and free of internal secondary structure, and avoid complementation at 3'-ends within each primer and with each other. A final concentration of 200 nM per primer is effective for most reactions. Optimal results may require a titration of primer concentrations between 100 and 500 nM.

Template Specifications

The target template for SYBR real-time PCR is plasmid DNA (10 to 10^7 copies), genomic DNA (100 pg to 1 μg), or cDNA (generated from 1 pg to 100 ng of total RNA). For best results, the amplicon size should be limited to 80 – 250 bp in size.

Magnesium Concentration

The 2×SYBR Green Reaction Mix includes magnesium at a final concentration of 3 mM which has been confirmed to be work well for most targets in restrict lab experiments. However, the optimal concentration may range from 3 to 6 mM. If necessary, use the separate tube of 25mM magnesium chloride to increase the magnesium concentration.

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Performing Real-Time PCR, continued

ROX Reference Dye

ROX Reference Dye can be included in the reaction to normalize the fluorescent reporter signal, for instruments that are compatible with that option. ROX is often supplied at a 25 µM concentration. Use the following table to determine the amount of ROX to use with a particular instrument:

Instrument	Amount of ROX per 40 µl reaction	Final ROX Conc.
ABI 7000, 7300, 7900HT	0.8 µl	500 nM
ABI7500 Mx3000P, Mx4000	0.08 µl	50 nM

NOTE

To accurately pipette 0.08 µl per reaction, dilute ROX 1:10 immediately before use and use 0.8 µl of the dilution.

Protocol for Instruments Using PCR Tubes or Plates

The following protocol uses components from the SYBR® Real-Time PCR Kit, and has been optimized for use with real-time qPCR instruments that use tubes or plates (see page 6 for instrument setting guidelines). Further optimization may be required.

1. The following table provides Master Mix volumes for a standard 40 µl reaction size. Note that preparation of a master mix is crucial in quantitative applications to reduce pipetting errors.

Component	Vol /1 rxn	Vol /100 rxns
2×SYBR RT-PCR Mix	20 µl	2000 µl
Taq DNA polymerase（5U/ µl）	0.4 µl	40 µl
PCR Forward Primer(10 µM)	0.8 µl	80 µl
PCR Reverse Primer(10 µM)	0.8 µl	80 µl
DNA Sample (1 pg to 1 µg total RNA)	4 µl	400 µl
dd H_2O	To 40 µl	To 4000 µl

[1]See the **Important** note on primer concentration on page 4.

[2]Add 1 pg to 1 µg DNA template to each reaction, the exact volume is feasible.

[3]Votex the SYBR RT-PCR Mix before use.

NOTE

Exposure to direct light for an extended period of time may result in loss of fluorescent signal intensity.

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2. **Three-Step Standard Cycling Program**
   95°C for 3 minute hold;
   40 cycles of:
   95°C, 15 seconds
   55°C, 30 seconds
   72°C, 30 seconds
   Melting Curve Analysis
   Program according to instrument instructions.

3. **Two-Step Standard Cycling Program**
   95°C for 3 minute hold;
   40 cycles of:
   95°C, 15 seconds
   60°C, 30 seconds
   Melting Curve Analysis
   Program according to instrument instructions.

Optimal temperatures and incubation times may vary for different target sequences.

1. Make sure that all components are at the bottom of the tube/plate; centrifuge briefly if needed.
2. See the table on the previous page for the amount/concentration of ROX to use for your specific instrument.
3. ***Melting curve analysis*** can identify the presence of primer dimers by their lower annealing temperature, compared to the amplicon. The presence of primer dimers is not desirable in samples that contain template, as it decreases PCR efficiency and obscures analysis and determination of cycle thresholds.
4. The formation of primer dimers most often occurs in no-template controls, where the polymerase enzyme is essentially idle, and in this case the quantitative analysis of the template samples is not affected.
5. Melting curve analysis of no-template controls can discriminate between primer dimers and spurious amplification due to contaminating nucleic acids in reagent components.

Appendix

Technical Service

World Wide Web

Visit the GenePharma Web Resource using your World Wide Web browser. At the site, you can:

- □ Download manuals in Adobe Acrobat (PDF) format
- □ Explore our catalog with full color graphics
- □ Get the scoop on our hot new products and special product offers
- □ Obtain citations for Invitrogen products
- □ Request catalog and product literature

The Genepharma URL is www.genepharma.com

Quality Control

The product is tested functionally by qRT-PCR using total HeLa RNA as template. Kinetic analysis must demonstrate a linear dose response with decreasing target concentration and detection of GAPDH mRNA in 1 pg of total HeLa RNA.

Contact Us

For more information or technical assistance, please call, write, fax, or email. Additional international offices are listed on our web page (www.genepharma.com).

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