How to Setup and Perform a qPCR Experiment.

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Abstract

GoTaq® qPCR Master Mix(a,b) is a reagent system for quantitative PCR (qPCR). The system contains a new fluorescent DNA-binding dye that often exhibits greater fluorescence enhancement upon binding to double-stranded DNA (dsDNA) than SYBR. Green I.

GoTaq® qPCR Master Mix is provided as a simple-to-use, stabilized 2X formulation that includes all components for qPCR except sample DNA, primers and water. This formulation, which includes a proprietary dsDNA-binding dye, a low level of carboxy-X-rhodamine (CXR) reference dye (identical to ROX™ dye), GoTaq. Hot Start Polymerase, MgCl2, dNTPs and a proprietary reaction buffer, produces optimal results in qPCR experiments. A separate tube of CXR Reference Dye is included for use with instruments that require a higher level of reference dye than that in the GoTaq® qPCR Master Mix.

Advantages of the GoTaq® qPCR Master Mix

Dye: The proprietary dye provides brighter dsDNA-dependent fluorescence than SYBR. Green I, with less PCR inhibition than SYBR® Green. The dye enables efficient amplification, resulting in earlier quantification cycle (C_q) values and an expanded linear range using the same filters and settings as SYBR® Green I. The CXR reference dye can be detected using the same filters and settings as those used for ROX™ dye.

Quantification cycle is formerly known as cycle threshold (C_t).

Polymerase/Buffer Formulation: GoTaq® Hot Start Polymerase contains full-length *Taq* DNA polymerase bound to a proprietary antibody that prevents polymerase activity at room temperature. Thermal activation is achieved by incubating the assembled reaction at 95°C for 2 minutes. The proprietary polymerase/buffer formulation accommodates extended cycle numbers (45–50 cycles) and is compatible with thermal cycling programs that require extended activation (95°C for 10 minutes).

Performance: You can expect reliable performance with minimal lot-to-lot variation: efficient, sensitive and linear qPCR amplification over a wide dynamic range.

GoTaq® qPCR Master Mix Protocol

If you are currently performing dye-based qPCR, the GoTaq® qPCR Master Mix can simply be substituted for your current master mix. For consistency within an experimental set, prepare a sufficient volume of reaction mix without template DNA for the DNA standard reactions and experimental sample reactions. The protocol for a 50µl reaction is outlined below. Component volumes may be scaled as appropriate. This protocol assumes that 20% of the reaction volume is DNA template (e.g., 10µl of DNA template added to 40µl of reaction mix). If the volume of DNA template is more or less than 10µl, adjust the volume of Nuclease-Free Water accordingly so that the final reaction volume is 50µl.

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Guidelines

Storage Conditions

GoTaq® qPCR Master Mix is shipped at –20°C. Upon arrival, store all components at –20°C, protected from light. For immediate use, components may be stored at 2–8°C, protected from light, for up to 3 months.

Spectral Properties

The proprietary dye in the GoTaq® qPCR Master Mix has spectral properties similar to those of SYBR® Green I: Excitation at 493nm and emission at 530nm. Instrument optical settings established for SYBR® Green I assays should be used with GoTaq® qPCR Master Mix. The CXR reference dye has the same spectral properties as ROX™: Excitation at 580nm and emission at 602nm. Use the instrument settings for ROX™ dye for reactions containing GoTaq® qPCR Master Mix.

Magnesium Chloride Concentration

The $MgCl_2$ concentration of the GoTaq® qPCR Master Mix has been determined to be optimal for performance. If desired, the $MgCl_2$ concentration may be adjusted using a PCR-grade stock solution (not provided).

Instrument Compatibility

GoTaq® qPCR Master Mix can be used with any real-time instrument capable of detecting SYBR® Green I or FAM™ dye. GoTaq® qPCR Master Mix contains a low level of CXR reference dye. If you are using any of the following instruments, supplement the GoTaq® qPCR reaction mix with 0.5µl of CXR Reference Dye per 50µl reaction.

- Applied Biosystems 7000 Sequence Detection System
- Applied Biosystems 7300 Real-Time PCR System
- Applied Biosystems 7700 Sequence Detection System
- Applied Biosystems 7900HT Real-Time PCR System

Materials

GoTaq® qPCR Master Mix for Dye-Based Detection A6001 by Promega

- ✓ qPCR primers by Contributed by users
- ✓ DNA template, positive control template standards by Contributed by users
- ✓ barrier pipette tips by Contributed by users
- ✓ sterile, nuclease-free, DNA-free tubes for reaction mix setup by Contributed by users
- ✓ optical multiwell reaction plates and adhesive fi lm covers by Contributed by users
- ✓ real-time thermal cycler by Contributed by users
- ✓ optional: sterile MgCl2 stock solution by Contributed by users
- ✓ alternative normalization dye, if required (e.g., fluorescein for BioRad instruments) by Contributed by users

Protocol

Step 1.

Prepare the standard DNA dilution series and experimental samples in nuclease-free water. Store on ice until use.

Step 2.

Carefully add 10µl of template (or water for no-template control reactions) to the appropriate wells of the reaction plate. Store plate at room temperature or on ice.

AMOUNT

10 µl Additional info: Template (or water)

Step 3.

Thaw the GoTaq® qPCR Master Mix at room temperature.

TEMPERATURE

20 °C Additional info: Thawing GoTaq® qPCR Master Mix

REAGENTS

GoTaq® qPCR Master Mix A6001 by Promega

Step 4.

Gently vortex to ensure it is adequately mixed. Take care to avoid foaming or extended exposure to light. Store on ice until use.

Step 5.

Prepare the reaction mix, without template DNA, by combining the reagents in the order listed in Table 1.

Table 1. Preparation of the Reaction Mix.

Component	Volume per 50µl Reaction	Final Concentration
GoTaq® qPCR Master Mix, 2X	25µl	1X
Nuclease-Free Water	to a final volume of 40µl	
upstream and downstream PCR primers	_____µl	0.2µM or 0.05–0.9µM each

NOTES

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See Guidelines for a list of instruments that require addition of the CXR Reference Dye.

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Some instruments such as the BioRad instruments require addition of a normalization dye (e.g., fl uorescein).

Step 6.

Gently vortex to mix. Take care to avoid foaming.

Step 7.

Carefully add the appropriate volume of reaction mix prepared in Step 5 (e.g., 40µl of reaction mix for a 50µl reaction) to the appropriate wells of the reaction plate prepared in Step 1. Take care to avoid cross contamination.

Step 8.

Seal the reaction plate, and centrifuge at low speed for 1 minute to bring all reaction components together and eliminate air bubbles.

Step 9.

Program the thermal cycler as per the manufacturer's instructions using the following guidelines:

a. Select SYBR® or FAM™ as the detection dye for the entire plate.

b. Select the ROX™ channel to detect CXR as the reference dye for the entire plate.

c. Select a standard or fast, two-step, 40-cycle qPCR and dissociation program. Please note that the cycling parameters given below are offered as a guideline and may be modified as necessary.

	# Cycles	Standard Cycling Program	Fast Cycling Program
Hot-Start Activation	1	95°C for 2 minutes	95°C for 2 minutes
Denaturation	40	95°C for 15 seconds	95°C for 3 seconds
Annealing/Extension		60°C for 60 seconds	60°C for 30 seconds
Dissociation	1	60–95°C	60–95°C

d. Designate that data will be collected during the annealing step of each cycle.

Step 10.

Place the plate into the instrument, and press “Start”.

When the run is complete, analyze the data using standard procedures.

Warnings

Please refer to the SDS (Safety Data Sheet) for hazard information.