FastGreen Q-PCR Master Mix (2X), High ROX

Q7002

Storage

Store at -20°C in the dark for long time storage.
Avoid multiple freeze-thaw cycles.

Contents

- Product Manual
- FastGreen Q-PCR Master Mix (2X), High ROX

Shipping Condition

Ship with dry ice.

Introduction

FastGreen Q-PCR Master Mix (2X), High ROX is a ready-to-use solution for performing accelerated quantitative real-time PCR on the Applied Biosystems: ABi 7000 PRISM, 7300, 7700, 7900HT, 7900 HT Fast, StepOne™ and StepOnePlus™.
The master mix includes Q-Fast Taq DNA polymerase that has been specifically engineered for faster replication, dNTPs, qGreen Fluorescent DNA binding Dye (substitute for SYBR Green I dye), ROX passive dye, and Mg^{2+} in an optimized PCR buffer. FastGreen Q-PCR Master contains unique heat-labile polymer that inactivate Taq DNA polymerase at room temperature and activate Taq DNA polymerase at 95°C in 10 sec. The unique mutant Taq and heat-labile polymer in combination with an optimized buffer ensures PCR specificity and sensitivity in accelerated processing time.

Primer Design Guideline

Amplified product size: 80–210 bp is recommended. (It is possible to amplify a target up to 300 bp in size.)

Primer length: 19 - 25 mer

GC content: 40 - 60% (45 - 55% is recommended.)

Tm: Tm values of forward and reverse primers must not be significantly different. 58–68°C

Sequence: The sequence should not be partially rich in any base in the whole sequence. Avoid including regions that have high GC or AT content, (especially at the 3'-end). Do not include polypyrimidine (serial T/C sequence). Do not include polypurine (serial A/G sequence).

Sequence of 3' end: The 3' terminus region should not have a high GC or AT content. We recommend that you choose a sequence with G or C at the 3' end and do not choose a sequence with T at the 3' end.
A complementary sequence of more than 3 bases should not exist within a primer or even between primer pairs. A primer pair should not have a complementary sequence of more than two bases at each 3' end.

Protocol - 2 Step Cycling Real-Time PCR

Step 1. Prepare a reaction master mix by adding the following components (except template DNA) for each 20 ul reaction to a tube at room temperature:
Gently mix the reactions without creating bubbles (do not vortex).
Centrifuge briefly if needed. Bubbles will interfere with fluorescence detection.

Components	Total 20ul
amfiSure qGreen Q-PCR Master Mix (2X), High ROX	10 ul
Forward primer, 10uM	0.4 ul (200 nM)
Reverse primer, 10uM	0.4 ul (200 mM)
Template DNA	as required (<50 ng)
Water, PCR certified	up to 20 ul

Note: **Template DNA**
High concentration of template may increase background fluorescence and reduce linearity of standard curves. For optimal quantitative results, use 10 pg-50 ng of genomic DNA or 1 pg - 5 ng of plasmid DNA per 20ul reaction.

For two-step RT-PCR, use either undiluted or diluted cDNA generated from up to 1 ug of total RNA. The volume of the cDNA (reverse transcription reaction product) should not exceed 10% of the final PCR volume (e.g. for a 20ul qPCR reaction, use up to 2ul of undiluted cDNA). For optimal quantative results, use 10 pg -100 ng of cDNA.

Step 2. Perform Real-Time PCR Reaction

2-step fast cycling protocol

Step	Temperature & Time	Cycle
Initial Denaturation	20 sec at 95 °C	1
Denaturation	3 sec at 95 °C	40
Annealing/Extension	30 sec at 60 °C (10-30 sec at 60 - 65 °C)	

Note: Design primers that amplify PCR products 80-210 bp in length.

Note: Design primers with a melting temperature (Tm) of 60-65°C, to take advantage of two-step cycling.

Note: Annealing/Extension time will be optimized between 10 - 30 seconds depending on cycler specification.

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Product Selection Guide

FastGreen Q-PCR Master Mix (2X), High ROX is compatible with Applied Biosystems and Stratagene Real-time PCR instruments. (Shown as table)

Instruments	
Bio-Rad: CFX96™/CFX384™, Opticon™, Chromo4™ **Roche**: LightCycler 480/1536, LightCycler 2.0 **Qiagen**: Rotor-Gene™ Q **Eppendorf**: MasterCycler **Corbett**: Rotor-Gene™ 3000, 6000 **Cepheid**: SmartCycler **Illumina**: Eco™	Q7000, Universal without ROX
Bio-Rad: iCycler iQ/iQ 5 , iCycler MyiQ™	Q7001, Fluorescein
Applied Biosystems: 7300, 7900 Fast, StepOne™, StepOnePlus™, ABI PRISM 7000, 7700 and 7900HT, 7900HT Fast	Q7002, High ROX Final ROX Conc 100nM
Applied Biosystems: 7500, 7500 Fast, ViiA™7, QuantaStudio series **Stratagene**: Mx3000P™, Mx3005P™, Mx4000™	Q7003, Low ROX Final ROX Conc 10nM

Important Parameters

Primer

- HPLC-purified primers are recommended to minimize loss in sensitivity due to nonspecific amplification.
- To maximize the sensitivity of the assay, use the lowest primer concentration determined not to compromise reaction efficiency (50-200 nM of each primer)
- Design primers that amplify PCR products 60-150 bp in length.
- Design primers with a melting temperature (Tm) of approximately 60°C, to take advantage of two-step cycling.
- If performing qRT-PCR, we recommend designing primers specifically for ampli -fication of cDNA derived from mRNA. This prevents amplification of contaminating genomic DNA and inaccurate quantification of mRNA.

dUTP

dUTP is included in the master mix to partially replaced dTTP in the accumulated PCR product, allowing for the option to prevent carryover comtamination between reactions. Uracil-DNA Glycosylase (UDG) pre-treatement of the reaction removes all dU-containing amplicons carried over from previous reactions.

Note: UDG is not included in the FastGreen Q-PCR Master Mix and must be purchased separately.

Related Product

Description	Cat No
amfiSure qGreen Q-PCR Master Mix (2X), without ROX	Q5600
amfiSure qGreen Q-PCR Master Mix (2X), Fluorescein	Q5601
amfiSure qGreen Q-PCR Master Mix (2X), High ROX	Q5602
amfiSure qGreen Q-PCR Master Mix (2X), Low ROX	Q5603
amfiSure Probe Q-PCR Master Mix (2X), without ROX	Q5700
amfiSure Probe Q-PCR Master Mix (2X), Fluorescein	Q5701
amfiSure Probe Q-PCR Master Mix (2X), High ROX	Q5702
amfiSure Probe Q-PCR Master Mix (2X), Low ROX	Q5703
amfiRivert cDNA Synthesis Platinum Master Mix	R5600
amfiRivert Reverse Transcriptase, 200 units/ul	A1202
amfiRivert Platinum One cDNA Master Mix (4X)	R6100
amfiRivert Sensi cDNA Master Mix (4X)	R6200
amfiRivert 4G cDNA Master Mix (4X)	R6300
PureXtract RNAsol, Trizol equivalent	R6101
gRazor, Genomic DNA Removal Enzyme Mix (5X)	R58000
RNazor, RNase Decontamination Solution	R7000
dNTP Mixtures, 10mM each, > 99% by HPLC	D0610
Water, DEPC Treated for RNA application	W0805
Water, PCR Certified	W0806
Agarose, Sepro	A0224
iVDye 50bp DNA Ladder	V1001
iVDye 100bp DNA Ladder	V1002
iVDye 1Kb DNA Ladder	V1003
100bp PCR Ranger DNA Marker, 100bp-to-3kb	D1108
1Kb PCR Ranger DNA Marker, 750kb-to-20kb	D1109

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Troubleshooting

Problems	Causes	Solutions
Positive signal in no-template control (NTC) Presence of secondary, nonspecific peak in melt curve of sample	Reasons for a positive signal in a NTC and/or nonspecific amplification are often due to multiple factors that include: 1. Contamination 2. Primer-dimer formation due to: - Incorrect cycling protocol - Sub-optional primer annealing temperature (often due to differences between qPCR systems) - Primer and/or template degradation. Primers should be stored and diluted in 10mM Tris-HCl (pH 8.0. 8.5), not in PCR-grade water) - Sub-optimal primer synthesis - Sub-optimal primer design	Perform melt curve analysis (or run qPCR products on a gel) to determine if the product is specific or nonspecific (primer-dimer). If NTC contains a specific product, the assay is contaminated: - Discard all reagents, clean all pipettes and surfaces and prepare fresh stocks of primer, etc. Note: The increased sensitivity of FastGreen qGreen Q-PCR Master Mix may result in the detection of low levels of contamination in assays considered contaminant -free when using competitor kits containing wild-type Taq DNA polymerase. If the NTC and /or sample contains nonspecific product, assay optimization may be required: - 30 sec combined annealing/extention time is recommended for most assays. Longer times may result in nonspecific amplification. - Increase the combined annealing/extention temperature in increments of 3°C. - Decrease primer concentration. - Resynthesize or redesign primers. HPLC purified primers are recommended for low copy number detection, and often results.
Low fluorescence intensity	Incorrect handling Incorrect concentration of ROX reference dye	qGreen dye is light sensitive; avoid exposure to light and repeated freeze-thaw cycles. Always thaw and mix solutions thoroughly before use. If the incorrect concentration of ROX reference dye is added to the master mix, the normalized signal may be lower than expected (if too much ROX has been added), or higher than expected (if too little ROX has been added). If using ABI instrumentation, analysis of the raw signal can always be performed with the ROX filter switched off.
Melting temperature of specific product is different from competitor kit	Differences in the buffer composition (e.g. salt concentration) of qPCR master mixes.	Differences in master mix formulation may affect the melting temperature of the products slightly. A particular DNA fragment will melt at a higher temperature in a reaction buffer containing a higher salt concentration.