amfiSure qGreen Q-PCR Master Mix (2X), Low ROX

Q5603

Storage

Store at -20°C in the dark for long time storage or at 4°C for up to one month. Avoid multiple freeze-thawing.

Contents

- Product Manual
- amfiSure qGreen Q-PCR Master Mix (2X), Low ROX
- Water, PCR Certified

Introduction

amfiSure qGreen Q-PCR Master Mix (2X) is a ready-to-use solution for quantitative real-time PCR and two-step real-time RT-PCR on most real-time PCR machines. The master mix includes amfiSure Hot-Start Taq DNA polymerase, dNTPs, qGreen Fluorescent DNA binding Dye (substitute for SYBR® Green I dye) and rox passive dye in an optimized PCR buffer. amfiSure Hot-Start Taq DNA polymerase in com -bination Primers with an optimized buffer ensures PCR specificity and sensitivity. qGreen Fluorescent intercalating dye allows for DNA detection and analysis without using sequence-specific probes. dUTP is included in the mix for optional carryover contamination control using uracil-DNA glycosylase (UDG). The use of amfiSure qGreen Q-PCR Master Mix in real-time PCR ensure reproducible, sensitive and specific quantification of genomic, plasmid, viral and cDNA templates.

Protocol

Step 1. Prepare a reaction master mix by adding the following components (except template DNA) for each 20ul 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), Low ROX	10 ul
Forward primer, 10uM	0.4 ul (200 nM)
Reverse primer, 10uM	0.4 ul (200 nM)
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 quantitative results, use 10 pg -100 ng of cDNA.

Step 2. Perform Real-Time PCR Reaction

Two-step cycling protocol

Step	Temperature & Time	Cycle
Optional UDG pre-treatment	2 min at 50 °C	1
Initial Denaturation note2	3 min at 95 °C	1
Denaturation	10 sec at 95 °C	40
Annealing/Extension	30-60 sec at 60 °C	

Three-step cycling protocol

Step	Temperature & Time	Cycle
Optional UDG pre-treatment	2 min at 50 °C	1
Initial Denaturation note2	3 min at 95 °C	1
Denaturation	10 sec at 95 °C	40
Annealing	20 sec at 60 °C	
Extension	30 sec at 72 °C	

Note 2: Initial Denaturation
20 sec at 95°C sufficient time for enzyme activation; however, optional denaturation of complex targets (genomic DNA) may require up to 3-5 min denaturation.

Fast 2-step cycling protocol with cDNA

Step	Temperature & Time	Cycle
Optional UDG pre-treatment	2 min at 50 °C	1
Initial Denaturation note2	3 min at 95 °C	1
Denaturation	5 sec at 95 °C	40
Annealing/Extension	20-30 sec at 60 °C	

Fast Three-step cycling protocol with cDNA

Step	Temperature & Time	Cycle
Optional UDG pre-treatment	2 min at 50 °C	1
Initial Denaturation note2	20 sec at 95 °C	1
Denaturation	5 sec at 95 °C	40
Annealing	15 sec at 60 °C	
Extension	10 sec at 72 °C	

Note 2: Initial Denaturation
20 sec at 95°C sufficient time for enzyme activation; however, optional denaturation of complex targets (genomic DNA) may require up to 3-5 min denaturation.

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Q5603

Product Selection Guide

amfiSure qGreen Q-PCR Master Mix (2X), low ROX is compatible with Bio-Rad, Roche, Qiagen, Eppendorf, Corbett, Cepheid, and Illumina Real-time PCR instruments. (Shownn 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™	Q5600, Universal without ROX
Bio-Rad: iCycler iQ/iQ 5 , iCycler MyiQ™	Q5601, Fluorescein
Applied Biosystems: 7300, 7500 Fast, StepOne™, StepOnePlus™, ABI PRISM 7000, 7700 and 7900HT	Q5602, High ROX Final ROX Conc 100nM
Applied Biosystems: 7500, ViiA™7, QuantaStudio™ 12K Flex **Stratagene**: Mx3000P™, Mx3005P™, Mx4000™	Q5603, Low ROX Final ROX Cocn 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: UDF is not included in the amifiSure qGreen Q-PCR Master Mix and must be purchased separately.

Product Selection Guide

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
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)	R5800
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 ***amfiSure*** 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 form 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.