

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), without ROX
- Water, PCR Certified

ALL PRODUCTS SOLD BY GenDEPOT ARE INTENDED FOR RESEARCH USE ONLY UNLESS OTHERWISE INDICATED. THIS PRODUCT IS NOT INTENDED FOR DIAGNOSTIC OR DRUG PURPOSE

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 combination 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), without 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.

Product Selection Guide

amfiSure qGreen Q-PCR Master Mix (2X), without ROX is compatible with Bio-Rad, Roche, Qiagen, Eppendorf, Corbett, Cepheid, and Illumina 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™ | 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 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 (T_m) of approximately 60°C, to take advantage of two-step cycling.
- If performing qRT-PCR, we recommend designing primers specifically for amplification 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 replace dTTP in the accumulated PCR product, allowing for the option to prevent carryover contamination between reactions. Uracil-DNA Glycosylase (UDG) pre-treatment of the reaction removes all dU-containing amplicons carried over from previous reactions.

Note: UDF is not included in the amfiSure qGreen Q-PCR Master Mix and must be purchased separately.

Product Selection Guide

| Description | Cat No |
|---|--------|
| 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 |
| 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 |

★ Troubleshooting

| Problems | Causes | Solutions |
|--|---|--|
| <p>Positive signal in no-template control (NTC)</p> <p>Presence of secondary, nonspecific peak in melt curve of sample</p> | <p>Reasons for a positive signal in a NTC and/or nonspecific amplification are often due to multiple factors that include:</p> <ol style="list-style-type: none"> 1. Contamination 2. Primer-dimer formation due to: <ul style="list-style-type: none"> - Incorrect cycling protocol - Sub-optimal 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 | <p>Perform melt curve analysis (or run qPCR products on a gel) to determine if the product is specific or nonspecific (primer-dimer).</p> <p>If NTC contains a specific product, the assay is contaminated:</p> <ul style="list-style-type: none"> - Discard all reagents, clean all pipettes and surfaces and prepare fresh stocks of primer, etc. <p>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.</p> <p>If the NTC and /or sample contains nonspecific product, assay optimization may be required:</p> <ul style="list-style-type: none"> - 30 sec combined annealing/extension time is recommended for most assays. Longer times may result in nonspecific amplification. - Increase the combined annealing/extension 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 | <p>Incorrect handling</p> <p>Incorrect concentration of ROX reference dye</p> | <p>qGreen dye is light sensitive; avoid exposure to light and repeated freeze-thaw cycles. Always thaw and mix solutions thoroughly before use.</p> <p>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.</p> |
| 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. |

NOTICE TO PURCHASER: LIMITED LICENSE

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,079,352, 5,789,224, 5,618,711, 6,127,155, 5,677,152, 5,773,258, 5,407,800, 5,322,770, 5,310,652, 5,994,056, 6,171,785, and claims outside the US corresponding to US Patent No. 4,889,818. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim (such as apparatus or system claims in US Patent No. 6,814,934) and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.