LIFESCT
Life Scientists' Service Center
Email: support@lifesct.com
Tel: 240-715-2985 Fax: 240-252-7376
9610 Medical Center Drive, Rockville, MD 20850

LiQuant™ Universal Green qPCR Master Mix

Cat. #: M0016 Size: 500/2500 rxns

Introduction

Liquant™ Universal Green qPCR Master Mix, protected by LiQuant Taq DNA Polymerase via an antibody-modified hot-start activation technique, is specially designed for SYBR Green I based quantitative PCR (qPCR). Unique factors (i.e. the specificity-promoting Exactor™) in the optimized buffer system of Liquant™ Universal green qPCR Master Mix significantly improve its sensitivity and specificity. This kit contains unique ROX Passive Reference Dye and is applicable for all Real-time PCR instruments, including instrument that needs no ROX dye, low ROX dye, or high ROX dye. The mix is prepared at 2× reaction concentration and can be directly used for robust and low-template qPCR with high sensitivity, specificity, and reliability.

Package Information

Components	M0016-05 (500 rxns)	M0015-25 (2500 rxns)
2× Liquant™ Universal green qPCR Master Mix	1.25 ml × 4	1.25 ml × 20

Storage

This mix can be stored at -20°C (protected from light) for 12 months or stored at 4°C (protected from light) for 6 months.

Application

This kit contains unique ROX Passive Reference Dye and is applicable for all Real-time PCR instruments, including instrument that needs no ROX dye, low ROX dye, or high ROX dye.

Applied Biosystems 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast; StepOne™, StepOnePlus™, 7500, 7500 Fast, ViiA™ 7; Bio-Rad CFX96 ™, CFX384™, iCycler iQ™, iQ™5, MyiQ™, MiniOpticon™, Opticon®, Opticon 2, Chromo4™; Qiagen/Corbett Rotor-Gene® Q, Rotor-Gene® 3000, Rotor-Gene® 6000; Stratagene MX4000™, MX3005P™, MX3000P ™; Eppendorf Mastercycler® ep realplex, realplex 2s; Roche Applied Science LightCyclerTM 480; and other Real-time PCR instruments.

Protocol

Note:

1. Aliquot reagents after the first use to avoid repeated freeze-thaw cycles.

2. White precipitation may appear during the thawing of the Liquant™ Universal green qPCR Master Mix. Before use, dissolve the precipitation by incubating at room temperature and gently flipping the tube. Mix the solution thoroughly every time before pipetting.

1. Prepare the reaction solution as follows:

2× Liquant™ Universal Green qPCR Master Mix	10 µl
Primer 1 (10 µM)	0.4 µl
Primer 2 (10 µM)	0.4 µl
Template DNA/cDNA	x µl
ddH_2O	To 20 µl

* For each component, the volume of can be adjusted according to the following principle:

a. The final concentration of primer is usually 0.2 µM, and if necessary, it can be adjusted between 0.1 µM and 1.0 µM.

b.The accuracy of template volumes impacts significantly on the qPCR results, due to the high sensitivity of LiQuant™ Universal Green qPCR Master Mix. Therefore, to improve experimental repeatability, it is recommended to dilute the template and pipet 2 µl-5 µl to the reaction system.

c. The volume of template (i.e. undiluted template) should be ≤ 1/10 of total volume.

2. Place the sample in a qPCR instrument and run the following program for qPCR:

Stage 1	Pre-denaturation*	Reps: 1	95°C	30 sec
Stage 2	Denaturation Annealing + Extension	Reps: 40	95°C 60°C	3-10 sec** 10-30 sec**
Stage 3	Melting Curve***	Reps: 1	95°C 60°C 95°C	15 sec 60 sec 15 sec

* Pre-denaturation at 95°C for 30 sec is suitable for most amplification. However, it could be prolonged to 3 min for templates with complicated structures.

** Denaturation for 10 sec is suitable for Standard PCR; denaturation for ≥ 3 sec is suitable for Fast PCR.

** Extension for 30 sec is suitable for standard PCR. For fast PCR, for amplicons ≤ 200 bp, the extension time should be ≥ 10 sec, while for amplicons > 200 bp, the extension time is recommended to prolong to 30 sec.

*** Program for melting curve may vary qPCR instruments. Please select the default melting curve program of the instrument used.

Optimizing Reaction System

Features of a good qPCR system includes (list in order of importance) a single peak in melting curve (indicating high amplification sensitivity), an e value close to 100% (indicating high amplification efficiency), and a low Ct value (indicating high amplification efficiency). If failed to get good qPCR performance using the default qPCR program, optimize the reaction system to improve the amplification sensitivity and efficiency according the following guidelines:

1. **Relationship between primer concentration and qPCR performance**: when the final concentration of primer ranges from 0.1 µM to 1.0 µM, increasing the primer concentration will lead to decrease in amplification specificity and improvement in amplification efficiency.

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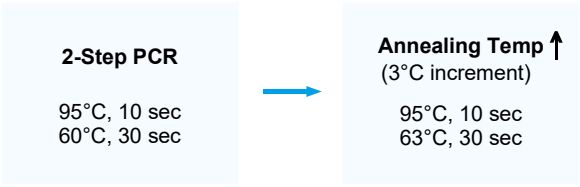
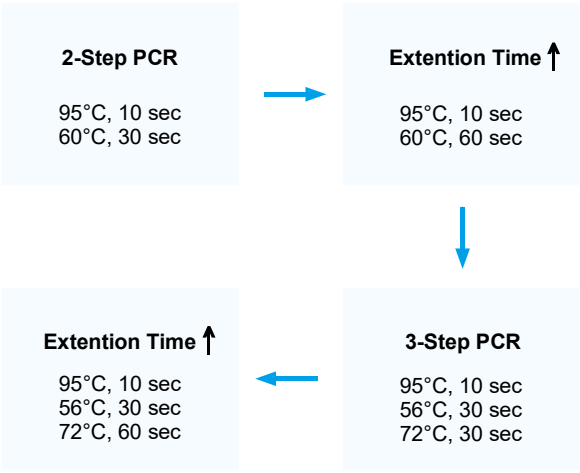
2. **Relationship between qPCR program and performance:**

To improve specificity, select a 2-step PCR program or increase the annealing temperature.



To improve efficiency, increase extension time or switch to a 3-step PCR program.



3. **Pre-denaturation time:** Pre-denaturation at 95°C for 30 sec is suitable for most templates. Extend the pre-denaturation to 3 min for template DNA with complicated structures.

Trouble Shooting

1. Abnormal shape of amplification plot

a. Rough amplification plot: Caused by system rectification due to weak signal. Elevate the template concentration and repeat the reaction.

b. Broken or downward amplification plot: Concentration of templates is too high. The end value of the baseline is bigger than Ct value. Decrease the end of the baseline (Ct value - 4) and re-analyze the data.

c. Amplification plot goes downward suddenly: Bubbles left in the tube break up when the temperature rises, shown as sudden decrease of the fluorescence value. Spin briefly and check closely if there are bubbles left before PCR.

2. No amplification plot

a. Insufficient cycling: The cycling number is set to be 40. Cycling with too many cycles leads to excessive background and reduces the data reliability.

b. Signals are not read during cycling: In 2-step PCRs, read signals during annealing and extension. In 3-step PCRs, read signals druing extension.

c. Primers are degraded: Test the integrity of primers (i.e. after long-term storage) using PAGE electrophoresis.

d. Low template concentration: Reduce the dilution fold and retry. For target gene with unknown expression level, begin without dilution in template.

e. Degradation of templates: Prepare new templates and repeat the PCR.

3. Ct value is two high

a. Low amplification efficiency: Optimize the PCR system (i.e. try 3-step PCR or re-design the primers).

b. Low template concentration: Reduce the dilution fold and retry. For target gene with unknown expression level, begin without dilution in template.

c. Degradation of templates: Prepare new templates and repeat the PCR.

d. The amplicon is too long: The recommended amplicon size is within 80 bp-150 bp.

e. PCR inhibitors in the system: Usually brought in when adding templates. Increase the dilution folds or prepare new templates ,and then retry.

4. Amplification observed in negative control

a. Contaminated reagents or water: Use new reagents or water and retry. Prepare the reaction system in a clean bench.

b. Primer dimers: It's normal to observe amplification of primer dimers in negative control after 35 cycles, which can be identified in the melt curve.

5. Poor fitness of the standard curve using linear regression in absolute qPCR

a. Deviations of pipetting volumn: Dilute the templates and increase the pipetting volume accordingly.

b. Degradation of standards: Prepare new standards and retry.

c. High template concentration: Increase the dilution fold.

6. Multiple peaks in melting curve

a. Unoptimized primers: Design new primers according to "Primer Designing Notes".

b. High primer concentration: Decrease the primer concentrations.

c. Contamination of genomic DNA in cDNA template: Prepare new cDNA templates with out Genomic DNA.

7. Poor reproducibility

a. Inaccurate pipetting volume: Use a more accurate pipettor, increase the pipetting volume by increasing the reaction volumn and diluting the templates.

b. Difference in temperature control between wells in qPCR instrument: Maintain the instruments periodically.

c. Low template concentration: The lower the template concentration, the worse the reproducibility. Decrease the dilution fold or increase the volume.