ChamQ SYBR® qPCR Master Mix (Without ROX)

Catalog # Q321

Version 6.1

Vazyme biotech co., ltd.

Introduction

The Vazyme ChamQ SYBR® qPCR Master Mix, protected by Champagne Taq DNA Polymerase (Vazyme, #P122) 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 ChamQ SYBR® qPCR Master Mix significantly improve its sensitivity and specificity. 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.

Contents of Kits

Components	Q321-01 (125 rxn / 20 μl reaction)	Q321-02 (500 rxn / 20 μl reaction)	Q321-03 (2,500 rxn / 20 μl reaction)
2× ChamQ SYBR qPCR Master Mix [a]	1.25 ml	1.25 ml × 4	Q321-02 × 5

a. Contains dNTPs, Mg^{2+}, Champagne Taq DNA Polymerase, SYBR Green I, etc.

Application

This kit doesn't contain ROX reference dye that is used to rectify the error of fluorescence signals between different wells. Therefore, this kit is applicable for Real-time PCR instruments that need no ROX dye.

Real-Time PCR Instruments DO NOT USE ROX Reference Dye	Bio-Rad CFX96™, CFX384™, iCycler iQ™, iQ™5, MyiQ™, MiniOpticon™, Opticon®, Opticon 2, Chromo4™; Cepheid SmartCycler®; Eppendorf Mastercycler® ep realplex, realplex 2 s; Illumina Eco qPCR; Qiagen/Corbett Rotor-Gene® Q, Rotor-Gene® 3000, Rotor-Gene® 6000; Roche Applied Science LightCycler™ 480; Thermo Scientific PikoReal Cycler, etc.

Storage

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

Quality Control

No activity of exonuclease, endonuclease, or nucleic acid was detected in all components.

Functional Assay: Human B2M gene was detected with 100 ng Hela cDNA as templates using this mix. The amplification efficiency was between 0.95 and 1.05. The Ct value at 10^{-5}-fold dilution was ≤ 35.

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 ChamQ SYBR® 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× ChamQ SYBR qPCR Master Mix	10.0 μl
Primer1 (10 μM)	0.4 μl
Primer2 (10 μM)	0.4 μl
Template DNA/cDNA	x μl
ddH_2O	To 20.0 μl

Note: 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 ChamQ SYBR® qPCR Master Mix. Therefore, to improve experimental repeatability, it is recommended to dilute the template and pipet 2-5 μl to the reaction system.

c. The size of the amplicon should be within the range of 80 bp-150 bp.

d. 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 [a]	Reps: 1	95℃	30 sec
Stage 2	Denaturation	Reps: 40	95℃	10 sec
	Annealing + Extension [b]		60℃	30 sec
Stage 3	Melting Curve [c]	Reps: 1	95℃	15 sec
			60℃	60 sec
			95℃	15 sec

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

b. Extension for 30 sec is suitable for amplicons ≤ 300 bp. It is recommended to prolong extension to 60 sec for amplicon > 300 bp.

c. 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.

2. Relationship between qPCR program and performance:

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

2-Step PCR	→	Annealing Temp ↑ (3℃ increment)
95℃, 10 sec 60℃, 30 sec	→	95℃, 10 sec 63℃, 30 sec

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

2-Step PCR	→	Extention Time ↑	→	3-Step PCR	→	Extension Time ↑
95℃, 10 sec 60℃, 30 sec	→	95℃, 10 sec 60℃, 60 sec	→	95℃, 10 sec 56℃, 30 sec 72℃, 30 sec	→	95℃, 10 sec 56℃, 30 sec 72℃, 60 sec

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

Primer Designing Notes

1. The amplicon size should be 80 bp-150 bp.
2. The primer length should be17 bp-25 bp.
3. Avoid GC-rich and AT-rich region at the 3'-end of the primer.
4. Choose C or G, instead of T, as the last base of the 3'-end of the primer.
5. Difference in Tm value between the forward and reverse primer should be ≤ 1℃. Tm values of primers should be with 60℃-65℃ (calculated with Primer 5).
6. GC content of the primers should be within the range of 40%-60% or 45%-55% as preferred.
7. A, G, C and T should be distributed as equally as within the primer. Avoid using GC- or TA-rich regions.
8. Avoid (self-)matching of ≥ 8 bases between all primers. At the 3'-ends, avoid matching of 3 bases between the forward and reverse primers.
9. Analyze the primers using the BLAST program on NCBI to eliminate the possibility of non-specific amplification.

Troubleshooting

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.