AceQ qPCR SYBR® Green Master Mix (Without ROX)

Catalog # Q121

Version 6.1

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Introduction

The Vazyme AceQ SYBR® qPCR Master Mix utilizes a special performance-enhanced AceTaq DNA Polymerase (Vazyme, #P401) protected via a chemically-modified hot-start activation technique to perform SYBR Green I based quantitative PCR (qPCR). The optimized buffer system of AceQ SYBR® qPCR Master Mix significantly improves the 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	Q121-01 (125 rxn/20 μl/rxn)	Q121-02 (500 rxn/20 μl/rxn)	Q121-03 (2,500 rxn/20 μl/rxn)
2× AceQ qPCR SYBR® Green Master Mix (Without ROX) [a]	1.25 ml	1.25 ml × 4	Q121-02 × 5

a. Contains AceTaq® DNA Polymerase, dNTP Mix, Mg^{2+}, 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℃ and protected from light.

Protocol

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

2. Mix thoroughly before use by gently inverting the tube upside-down for several times. **DO NOT vortex!** A brief centrifugation to remove air bubbles before use is highly recommended.

3. Protect the mix from light during storage and usage.

4. Prepare the reaction system in a clean bench with filtered tips, sterilized tubes and clean pipettors to eliminate the possible contaminations from air.

5. It is highly recommended to run at least three replicates for each sample.

1. Prepare the reaction solution as follows:

2× AceQ qPCR SYBR® Green Master Mix (Without ROX)	10.0 μl
Primer 1 (10 μM)	0.4 μl
Primer 2 (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 AceQ SYBR® qPCR Master Mix. Therefore, to improve experimental repeatability, it is recommended to dilute the template and pipet more volumn to the reaction system.

c. The size of the amplicon should be within the range of 100 bp-500 bp or 100 bp-200 bp as preferred.

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℃	5 min
Stage 2	Denaturation	Reps: 40	95℃	10 sec
	Annealing + Extension		60℃	30 sec
Stage 3	Melting Curve	Reps: 1	95℃	15 sec
			60℃	60 sec
			95℃	15 sec

a. The AceQ SYBR® qPCR Master Mix contains a chemically-modified hot-start DNA polymerase. The pre-denaturation should be at 95℃ for at least 5 min. The pre-denaturation time could be prolonged to 10 min for template DNA with complicated structures.

Primer Designing Notes

1. The amplicon size should be 100 bp-150 bp.

2. The primer length should be 17 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 continuous structures of T/C and A/G.

9. Avoid (self-)matching of ≥ 8 bases between all primers. At the 3'-ends, avoid matching of ≥ 3 bases between the forward and reverse primers.

10. 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 100 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.