AceQ qPCR Probe Master Mix

Catalog # Q112

Version 6.1

Vazyme biotech co., ltd.

Introduction

The AceQ qPCR Probe Master Mix is designed for probe-based qPCR. The hot-start AceTaq DNA Polymerase (Vazyme, #P401) and an optimized buffer significantly improve its sensitivity and specificity for qPCR. This kit is a 2× premixed ready-to-use reagent and can be used for robust and low-template qPCR with high sensitivity and specificity. When using this product for qPCR reactions, reliable standard curve can be obtained in a wide range and accurate qualification of target genes can be achieved with high repeatability and reliability.

Contents of Kits

Components	Q112-01 (125 rxn / 20 μl reaction)	Q112-02 (500 rxn / 20 μl reaction)	Q112-03 (2,500 rxn / 20 μl reaction)
2× AceQ qPCR Probe Master Mix [a]	1.25 ml	1.25 ml × 4	
50× ROX Reference Dye 1 [b]	50 μl	200 μl	**Q112-02** × 5
50× ROX Reference Dye 2 [b]	50 μl	200 μl	

a. Contains dNTP Mix, Mg^{2+}, and AceTaq DNA Polymerase.

b. Used to rectify the error of fluorescence signals between different wells. Select the appropriate ROX reference dye according to the Real-time PCR instrument used:

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.
USE ROX Reference Dye 1 (1×)	Applied Biosystems 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast; StepOne™, StepOnePlus™.
USE ROX Reference Dye 2 (1×)	Applied Biosystems 7500, 7500 Fast, ViiA™7; Stratagene MX4000™, MX3005P™, MX3000P™.

Storage

Store at -20℃ and protect from light.

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

Protocol (using ABI StepOnePlus™)

1. Prepare the reaction solution as follows:

2× AceQ qPCR Probe Master Mix	10.0 μl
Primer 1 (10 μM)	0.4 μl
Primer 2 (10 μM)	0.4 μl
TaqMan Probe (10 μM)	0.2 μl
50× ROX Reference Dye 1	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 final concentration of TaqMan Probe can be adjusted between 50 nM and 250 nM.

c. The accuracy of template volumes impacts significantly on the qPCR results, due to the high sensitivity of AceQ qPCR Probe Master Mix. To improve repeatability, it is recommended to use a 50 μl system and diluted template solution (i.e. 5 μl/sample) for qPCR.

d. The volume of undiluted cDNA template should be ≤ 1/10 of total volume.

2. Put 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 [b]		60℃	30 sec

Note: a. Pre-denaturation at 95℃ for 5 min is suitable for most amplification. However, it could be prolonged to 10 min for templates with complicated structures.

b. The extension time varies between different qPCR instruments used. For ABI 7700 and 7900HT, the extension time should be ≥ 30 sec; for ABI 7000 and 7300, the extension time should be ≥ 31 sec; for ABI 7500, ≥ 34 sec; and for ABI StepOne Plus™, ≥ 10 sec.

Primer Designing Notes

1. The primer length should be 17 bp-25 bp.
2. GC content of the primers should be within the range of 40%-60% or 45%-55% as preferred.
3. A, G, C, and T should be distributed as equally as within the primer. Avoid using GC- or TA-rich regions.
4. Avoid continuous T/C and A/G structures in primer design.
5. Avoid using > 2 G or C at the last 5 bases of the 3'-end of primer.
6. Both the forward and reverse primers should be close to the region of TaqMan Probe, but should not overlap with it.

TaqMan Probe Designing Notes

1. The TaqMan Probe should be close to the regions of the forward and reverse primers, but should not overlap with them.
2. The length of TaqMan Probe should be 18 bp-40 bp.
3. Avoid continuous identical bases, especially GGGG or more.
4. At the 5'-ends, avoid using G.
5. The annealing temperature of probe should be 65℃-67℃.
6. If there is polymorphic locus in the sequence, make it lie in the middle of the probe.

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 too 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. 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.