AceQ qPCR Probe Master Mix

Q112

Version 22.1

Product Description

The AceQ qPCR Probe Master Mix is specially designed for probe-based qPCR. The core component Ace Taq DNA polymerase is chemically modified hot-start DNA polymerase, which is combined with an optimized buffer for qPCR can effectively inhibit nonspecific amplification, thus significantly improving the amplification efficiency, and is suitable for high-sensitivity qPCR. This product is a 2 × premixed reagent, which can obtain reliable standard curve in a wide quantitative area. The target gene can be accurately quantified and detected, with good repeatability and high reliability.

Components

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

a. It contains dNTP Mix, Mg^{2+}, and AceTaq DNA Polymerase, etc.

b. It is used to correct the error of fluorescence signals between different wells. Select the appropriate ROX Reference Dye according to the Real-time PCR instrument used:

No ROX Reference Dye	Bio-Rad CFX96, CFX384, iCycler iQ, iQ5, 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.
Add ROX Reference Dye 1 (1×)	Applied Biosystems 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast; StepOne, StepOnePlus.
Add ROX Reference Dye 2 (1×)	Applied Biosystems 7500, 7500 Fast, ViiA7; Stratagene MX4000, MX3005P, MX3000P.

Storage

Store at -30 ~ -15℃ and transport at ≤0℃.

Applications

It is apllicable for DNA amplification from various type of templates such as genomic DNA, cDNA, plasmid DNA and λDNA.

Notes

For research use only. Not for use in diagnostic procedures.

Experiment Process (Using ABI StepOnePlus)

1. Prepare the following mixture in a qPCR tube:

2 × AceQ qPCR Probe Master Mix	10 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	Up to 20 μl

The volume of each component in the reaction system can be adjusted according to the following principles:

▲ Generally, the final concentration of primer in the reaction system is 0.2 μM to obtain better amplification effect. When the reaction performance is poor, the primer concentration can be adjusted in the range of 0.1 - 1.0 μM.

▲ The final concentration of TaqMan Probe can be adjusted between 50 - 250 nM.

▲ Due to the high sensitivity of qPCR, the accuracy of template volume has a significant impact on qPCR results. In order to effectively improve the repeatability of the experiment, it is recommended to dilute the template and add it to the reaction system.

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

2. Run the following program for qPCR:

Stage 1	Initial Denaturation[a]	Rep: 1	95℃	5 min
Stage 2	Cycles[b]	Reps: 40	95℃	10 sec
			60℃	30 sec

a. AceTaq DNA Polymerase requires thermal activation to restore the enzyme activity. Please set the condition of initial denaturation at 95℃ for at least 5 min. If the GC content of the template is high, the time of initial denaturation can be extended to 10 min.

b. Please adjust the extension time according to the minimum time limit of data acquisition required by the Real-time PCR instrument 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, the extension time should be ≥34 sec; and for ABI StepOne Plus, the extension time should be ≥10 sec.

Primer Design Guidance

1. The ideal primer length is 17 - 25 bp. Primers that are too short are likely to result in reduced amplification efficiency. Primers that are too long are more likely to generate higher structures. Both of them will interfere with the accuracy of the quantitative results.
2. Control the GC content of the primers at 40% - 60%, and the optimum GC content is from 45% - 55%.
3. The Tm value of the primer should be greater than 60℃. Primer Premier 5 is recommended to calculate the Tm value.
4. The overall distribution of A, G, C, and T in the primer should be as uniform as possible. Avoid using regions with high GC or TA content, especially at the 3' end. Regions with uneven GC content must be avoided.
5. Try to avoid structures with consecutive T/C or A/G when designing primers.
6. The last five bases at the 3' end of the primer must not contain more than two G or C.
7. Ensure that the forward or the reverse primer is as close as possible to the probe sequence, but does not have overlap regions with the probe sequence.

TaqMan Probe Design Guidance

1. The probe sequence should be as close as possible to the forward or reverse primer, but overlap sequences must be avoided.
2. The probe length is typically 18 - 40 bp.
3. Avoid consecutive identical bases, especially four or more consecutive G.
4. Avoid using G at the 5' end of the probe.
5. The annealing temperature of the probe should be 65 ~ 67℃.
6. If there is polymorphic locus in the sequence, make it lie in the middle of the probe.

FAQ & Troubleshooting

◇Abnormal shape of amplification plot

①Rough amplification plot: The signal is too weak and generated after system correction. Increase template concentration and retry.

②Broken or downward amplification plot: The template concentration is too high and the baseline endpoint is greater than C_T value. Reduce the baseline endpoint (C_T value - 4) and repeat data analysis.

③Amplification plot goes downward suddenly: There are bubbles remaining in the reaction tube. Pay attention to centrifugation when processing samples and carefully check the reaction tube for any remaining bubbles before performing reaction.

◇No amplification plot

①Insufficient number of reaction cycles: In general, the number of cycles is set to 40, but it should be noted that too many cycles will increase too many background signals and reduce the reliability of the data.

②Confirm whether the signal acquisition step is set up in the program: The two-step amplification program generally sets the signal acquisition at the annealing and extension stage, while the three-step amplification program should set the signal acquisition at the 72℃ extension stage.

③Confirm whether the primers are degraded: Primers that have not been used for a long time should be tested for integrity using PAGE electrophoresis before use in order to rule out the possibility of degradation.

④Low template concentration: Reduce the dilution factor and repeat the test. In general, samples with unknown concentration should be started at the highest concentration.

⑤Template degradation: Prepare new template and retry.

◇C_T value appears too late

①Low amplification efficiency: Optimize the PCR system, then try the three-step amplification program or redesign the synthetic primers.

②Low template concentration: Reduce the dilution factor and repeat the test. In general, samples with unknown concentration should be started at the highest concentration.

③Template degradation: Prepare new template and retry.

④Long PCR products: The recommended length of PCR product is 80 - 150 bp.

⑤PCR inhibitors in the system: They are usually introduced along with the template. Increase the dilution factor or prepare new template and retry.

◇Amplification observed in negative control

①The reaction solution is contaminated: Replace the mix, ddH_2O, primers and probe, then repeat the test. The reaction system is prepared in ultra-clean worktable to reduce aerosol pollution.

◇Standard curve linearity is poor for absolute quantification

①Sample loading error: Increase the template dilution factor and the loading volume of sample.

②Standard product degradation: Prepare the standard again and repeat the test.

③Template concentration is too high: Increase the template dilution factor.

◇Poor experiment repeatability

①Inaccurate pipetting volume: Use higher performance pipette; increase the template dilution factor, and increase the sample loading volume.

②Differences in temperature control between wells in qPCR instrument: Calibrate the instrument regularly.

③Low template concentration: The lower the template concentration, the worse the repeatability. Reduce the template dilution factor or increase the volume of sample addition.