AceQ Universal Probe Master Mix V2

Q513-EN

Version 22.1

Product Description

AceQ Universal Probe Master Mix V2 is a probe-based reagent for qPCR. The core component AceTaq DNA Polymerase is a chemically modified hot-start DNA polymerase. Combined with optimized buffer for qPCR, it can effectively inhibit non-specific amplification, and significantly improve the amplification efficiency. It is suitable for high-sensitivity qPCR. This product is a 2 × master mix. A good standard curve can be obtained in a wide quantitative area, and the target gene can be accurately quantified and detected, with good repeatability and high reliability. This product contains a unique ROX Passive Reference Dye that is suitable for all qPCR instruments. The concentration of ROX does not need to be adjusted on different instruments.

Components

Components	Q513-EN02 (500 rxns/20 μl reaction)	Q513-EN03 (2,500 rxns/20 μl reaction)
2 × AceQ Universal Probe Master Mix V2*	4 × 1.25 ml	5 × Q513-EN02

* It contains dNTP Mix, Mg^{2+}, AceTaq DNA polymerase, Specific ROX Reference Dye.

Storage

Store at -30 ~ -15℃ and transport at ≤0℃. Keep away from light.

Applications

This product is suitable 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 Universal Probe Master Mix V2	10 μl
Primer 1 (10 μM)	0.4 μl
Primer 2 (10 μM)	0.4 μl
TaqMan Probe (10 μM)	0.2 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, and do not use ROX-labeled probes.

▲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 qPCR program as follows:

Stage 1	Contamination Digestion	Rep: 1	37℃	2 min
Stage 2	Initial Denaturation[a]	Rep: 1	95℃	5 min
Stage 3	Cycles[b]	Reps: 45	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 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℃.

FAQ & Troubleshooting

◇ Abnormal shape of amplification plot

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

② Broken 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.

③ Amplification 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

① Insufficient number of reaction cycles: In general, the number of cycles is set to 45, but it should be noted that too many cycles will increase too many background signals and reduce the reliability of the data.

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

③ Check if 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.

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

⑤ Template degradation: Prepare new template and retry.

◇ C_T value appears too late

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

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

③ Template degradation: Prepare new template and retry.

④ Extra long PCR products: The recommended PCR product length is 80 - 150 bp.

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

◇ Amplification observed in negative control

① Contaminated of reaction system: Replace with new mix, ddH_2O and primers to repeat the experiment.

◇ Standard curve linearity is poor for absolute quantification

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

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

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

◇ Poor experiment repeatability

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

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

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