AceQ Universal U+ Probe Master Mix V2

Catalog # Q513

Version 9.1

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Introduction

AceQ Universal U+ 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 expansion efficiency and is suitable for high-sensitivity qPCR reactions. The dUTP/UDG anti-pollution system was introduced to the mix to eliminate the influence of the product contamination on qPCR. The mix, prepared at 2 × reaction concentration, can be directly used for robust and low-template quantitative PCR with high sensitivity, specificity and reliability. The ROX Passive Reference Dye included in AceQ Universal U+ Probe Master Mix V2 is a particular dye which makes this Mix applicable to all qPCR instruments, with no need to adjust the Rox concentration for different instruments.

Package Information

Components	Q513-02 (500 rxn/20 μl reaction)	Q513-03 (2,500 rxn/20 μl reaction)
AceQ Universal U+ Probe Master Mix V2*	4 × 1.25 ml	5 × Q513-02

* Contain dNTP/dUTP Mix , Mg^{2+}, AceTaq DNA polymerase, Heat-labile UDG, Specific ROX Reference Dye.

Applicable qPCR Instrument

Applied Biosystems 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast; StepOne™, StepOnePlus™ 7500, 7500 Fast, ViiA™7;

Bio-Rad CFX96™, CFX384™, iCycler iQ™, iQ™5, MyiQ™, MiniOpticon™, Opticon®, Opticon 2, Chromo4™;

Qiagen/Corbett Rotor-Gene® Q, Rotor-Gene® 3000, Rotor-Gene® 6000;

Stratagene MX4000™, MX3005P™, MX3000P™;

Eppendorf Mastercycler® ep realplex, realplex 2 s;

Roche Applied Science LightCyclerTM 480 and other instrument;

▲This product contains ROX Passive Reference Dye, applicable to all QPCR instrument, do not need to adjust the Rox concentration for different instruments.

Storage

Store at -30˚C ~ -15˚C, and protected from light. Shipped at -20˚C to 4˚C.

Protocol (Using ABI AtepOnePlus™)

1. Prepare a reaction solution in a qPCR tube as follows:

2 × AceQ Universal SYBR Green qPCR 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
Template DNA/cDNA	x μl
ddH_2O	Up to 20.0 μl

The amount of each component in the reaction solution can be adjusted according to the following principles:

▲ 0.2 μM of primer final concentration is applicable for most cases. The concentration can be adjusted within 0.1 - 1.0 μM when amplification efficiency is not satisfactory.

▲ The final concentration of the probe can be adjust among 50 - 250 nM, do not use Probe labeled by ROX.

▲ The accuracy amount of template added has significant influence on the final quantitative results due to the extremely high sensitivity of qPCR reaction. It is recommended to dilute the template before use to improve the repeatability of experiment.

▲ If template is stock solution, the volume of template added should be ≤ 1/10 of total reaction volume.

2. Perform qPCR reaction at the following cycling conditions:

Stage 1	Pollutant digestion	Reps:1	37℃	2 min
Stage 2	Pre-denaturation	Reps:1	95℃	5 min
Stage 3	Cycling reaction	Reps: 45	95℃	10 sec
			60℃	30 sec

a. The AceTaq DNA Polymerase included in this mix is a hot start Taq DNA polymerase, so that the pre-denaturation stage should be set at 95℃ for at least 5 minutes. And it can be extend to 10 minutes if GC content of the template is higher.

b. Extension time may be adjusted according to the qPCR instruments used. For example, the extension time should be set to no less than 30 seconds when using ABI 7700 and 7900HT, 31 seconds when using ABI 7000 and 7300, 34 seconds when using ABI 7500, or 10 seconds when using ABI StepOnePlusTM .

Primer Designing Notes

1. The primer length is preferably 17 - 25 bp. Too short primers tend to cause a decrease in the efficiency of the expansion; too long primers can lead to an increase in higherorder structure of the primers. Both will affect the accuracy of the quantitative results.
2. The GC content of the primer should be controlled at 40% - 60%, preferably 45% - 55%.
3. The Tm value of the primer should be > 60℃. It is recommended to use the Primer Premier 5 for Tm calculation.
4. The overall distribution of primers A, G, C, and T should be as uniform as possible. Avoid using areas with high GC or TA content, especially at the 3' end, and avoid areas with uneven GC content.
5. Try to avoid the continuous structure of T/C or AVG when designing primers.
6. The last five bases of the 3' end of the primer cannot contain more than two G or C.
7. The forward or reverse primer should be as close as possible to the probe sequence, but it cannot overlap with the probe sequence.

TaqMan Probe Design Guide

1. The probe sequence should be as close as possible to the forward or reverse primer, but not overlap with it.
2. The probe length is generally 18 - 40 bp.
3. Avoid the consecutive identical bases, especially to avoid GGGG or more consecutive G appearances.
4. The 5’ end of the probe should avoid the use of base G.
5. The annealing temperature of the probe should be 65 - 67℃.

FAQ and Solutions

◇ Abnormal shape of amplification plot

① Rough amplification plot: It is caused by system rectification due to weak signal. Elevate the template concentration and repeat in the reaction.

② Broken or downward amplification plot: The 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.

③ Amplification plot goes downward suddenly: There are bubbles left in the reaction tube, which break up when the temperature rises, thus the instrument detects the sudden decrease of the fluorescence value. Spin briefly and check closely if there are bubbles left before reaction.

◇ No amplification plot

① Cycling number is insufficient: Generally the cycling number is set to be 40. But notice that too many cycles will result in excessive background, thereby reducing the reliability of the data.

② Check if there is signal collection procedure during cycling: in two-step program, signal collection is usually positioned at annealing and extension stage; for three-step program, signal collection should be positioned at 72℃ extension stage.

③ Check if the primers are degraded: Test the integrity of primers after long-term storage through PAGE electrophoresis to confirm the presence of primers in solution.

④ The concentration of templates is too low: Reduce the dilution fold and retry. For target gene with unknown expression level, begin without template dilution.

⑤ Degradation of templates: Prepare new templates and retry.

◇ Ct value appears too late (high)

① Low amplification efficiency: Optimize the reaction. Try three-step program or re-design primers.

② The concentration of templates is too low: Reduce the dilution fold and retry. For target gene with unknown expression level, begin without template dilution.

③ Degradation of templates: Prepare new templates and retry.

④ The amplicon is too long: The length of the amplicon is recommended to be within 100 bp - 200 bp.

⑤ There are PCR inhibitors in the reaction: They are usually brought in when adding templates. Increase the dilution folds or prepare new templates and retry.

◇ Ct value appears too late (high)

The reagents or water used is contaminated: Change new reagents, water, probe and retry.

◇ The linear relation of the standard curve is not satisfactory when performing absolute qualification

① Pipetting error: Dilute the templates to increase the pipetting volume.

② Degradation of standards: Prepare new standards and retry.

③ Too high template concentration: Increase the dilution fold.

◇ The linear relation of the standard curve is not satisfactory when performing absolute qualification

① The primers are not optimal: Design new primers according the design principles.

② Too high concentration of primers: Appropriately decrease the concentration of primers.

③ cDNA template is contaminated by genomic DNA: Prepare new cDNA templates.

◇ Experiment has low reproducibility

① Inaccurate pipetting volume: Use a more accurate pipettor, elevate the reaction volume, and dilute the templates to increase the pipetting volume.

② Difference in temperature control in different wells of qPCR instrument: Regularly maintain the instruments.

③ Too low template concentration: The lower the template concentration, the worse the repeatability. Decrease the dilution fold or increase the volume of template used.