AceQ Universal SYBR qPCR Master Mix

NB-54-0171-01
NB-54-0171-02

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Introduction

AceQ Universal SYBR qPCR Master Mix utilizes a special performance-enhanced Taq DNA polymerase protected via a hot-start activation technique, and optimized qPCR buffer system to perform SYBR Green I based quantitative PCR (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 SYBR Green qPCR Master Mix is a peculiar dye which makes this Mix applicable to all qPCR instruments. And amplification can be carried out as long as primers and templates are added, do not need to adjustthe Rox concentration for different instruments.

Contents of Kits

Components	NB-54-0171-01 (500 rxn /20 µl reaction)	NB-54-0171-02 (2,500 rxn /20 µl reaction)
2x AceQ Universal SYBR	4 x 1.25 ml	5 x NB-54-0171-01

* Contain dNTP, $Mg^{2+,}$ AceTaq DNA polymerase, SYBR I dye, ROX Passive Reference Dye

Applicable qPCR instruments

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.
Applied Biosystems 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast; StepOne™, StepOnePlus™ Applied Biosystems 7500, 7500 Fast, ViiA™7;
Stratagene MX4000™, MX3005P™, MX3000P™

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

Storage

Store at -20℃, and protected from light.

Protocol (Using ABI AtepOnePlus™)

1. **Prepare a reation solution in a qPCR tube as follows :**

2x AceQ Universal SYBR Green qPCR Master Mix	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

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

a. 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.

b. 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.

c. 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	Pre-denaturation	Reps:1	95℃	5 min
Stage 2	Cycling reaction	Reps: 40	95℃	10 sec
			60℃	30 sec
Stage 3	Melting curve	Reps: 1	95℃	15 sec
			60℃	60 sec
			95°C	15 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 .

Notes

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.

FAQs and Troubleshooting

◇ **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.

②Broken 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.

③Amplification 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.

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

③Degradation of templates: Prepare new templates and retry.

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

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

◇ **Apparent amplification can be observed in negative control**

① The reagents or water used is contaminated: Change new reagents or water and retry. The reaction should be set up in a clean bench to minimize contamination from the air.

② Appearance of primer dimer: it is normal that amplification occurs in negative control after 35 cycles. Analysis can be performed combining with the melt curve.

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

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

②Degradation of standards: Prepare new standards and retry.

③Too high template concentration: Increase the dilution fold.

◇ **Multimodal dissociation curve**

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

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

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

◇ **Experiment has low reproducibility**

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

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

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