AceQ Universal SYBR qPCR Master Mix

Q511

Version 23.1

Product Description

AceQ Universal SYBR qPCR Master Mix is a special premix for qPCR detection using SYBR Green I chimeric fluorescence method. The core component AceTaq DNA Polymerase is a chemically modified hot-start DNA polymerase. Combined with the optimal Buffer optimized for qPCR, nonspecific amplification can be effectively inhibited, thus significantly improving the amplification efficiency, which is suitable for high-sensitivity qPCR detection. This product is a dye-based 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	Q511-02 500 rxns (20 μl/rxn)	Q511-03 2,500 rxns (20 μl/rxn)
2 × AceQ Universal SYBR qPCR Master Mix*	4 × 1.25 ml	5 × Q511-02

* It contains dNTPs, Mg^{2+}, AceTaq DNA Polymerase, SYBR Green I, Specific ROX Passive Reference Dye, etc.

Storage

Store at -30 ~ -15°C and protect from light. Transport at ≤0°C.

Applications

It is applicable for DNA quantification from various type of templates such as genomic DNA, cDNA, plasmid DNA and λ DNA.

Applicable Instruments

Bio-Rad CFX96, CFX384, iCycler iQ, iQ5, MyiQ, MiniOpticon, Opticon, Opticon 2, Chromo 4;
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, ViiA7; Stratagene MX4000, MX3005P, MX3000P.

▲ This product utilizes a special ROX reference dye that is compatible with all qPCR instruments (no ROX calibration instrument, low concentration ROX calibration instrument and high concentration ROX calibration instrument). There is no need to adjust the ROX concentration on different instruments.

Notes

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

1. Avoid repeated freezing and thawing, so as not to cause the decrease of enzyme activity. If the amount of each use is small, it is recommended to aliquot Master Mix into small portions.
2. Please invert the Master Mix upside down several times to mix thoroughly. Do not vortex to avoid air bubbles, affecting quantitative results. The Master Mix is ready to use after mixing and centrifuging briefly. Gently pipette up and down during sample loading. If foam appears in the Master Mix due to careless operation, it needs to be centrifuged again before use.
3. As this product contains the fluorescent dye SYBR Green I, it should be stored away from light. Avoid strong light when preparing the reaction system.
4. This product has high detection sensitivity and is easily contaminated by aerosols in the air. Therefore, the reaction system should be prepared on an ultra-clean workbench. Please use sterilized pipette tips and reaction tubes during the preparation process. If conditions permit, it is recommended to use dedicated pipettes and filter tips.

Experiment Process (Using ABI StepOnePlus)

1. Prepare the following mixture in a qPCR tube

Components	Volume
2 × AceQ Universal SYBR 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 volume of each component in the reaction system can be adjusted according to the following principles:

▲ Generally, a good result can be obtained when the final concentration of primer in the reaction system is 0.2 μM. If the result is not as expected, the primer concentration can be adjusted between 0.1 - 1.0 μM.

▲ 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	Initial Denaturation[a]	Rep: 1	95℃	5 min
Stage 2	Cycling Reaction[b]	Reps: 40	95℃	10 sec
			60℃	30 sec
Stage 3	Melting Curve[c]	Rep: 1	95℃	15 sec
			60℃	60 sec
			95℃	15 sec

a. AceTaq DNA Polymerase requires thermal activation to restore the enzyme activity. Please set the condition of initial denaturation at 95°C 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: 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.

c. Different instruments have different melting curve acquisition procedures. Just use the default melting curve acquisition program.

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 40, 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°C extension stage in three-step amplification program.

③ Confirm 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.

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.

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

⑤ PCR 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

① Contaminated of reaction system: Replace with new mix, ddH_2O and primers to repeat the experiment. The reaction system should be prepared in clean bench to reduce aerosol contamination.

② Primer dimer: Carry out analysis in association with the melting curve.

◇ 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.

◇ Multiple peaks in melting curve

① Inappropriate primer design: Design and synthesize new primers according to the primer design principles.

② High primer concentration: Decrease the primer concentration.

③ cDNA template with contamination of genomic DNA: Prepare new cDNA templates.

◇ 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.