AceQ® Universal SYBR qPCR Master Mix

Cat# NB-54-0171-00 / -01 / -02

Product Description

AceQ Universal SYBR qPCR Master Mix is a special premix for qPCR reaction 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, the AceTaq DNA Polymerase can effectively inhibit non-specific amplification, thus significantly improving the amplification efficiency, and it is suitable for high sensitivity qPCR reaction. This product is a 2 × premix SYBR Green I premix containing the optimal concentration for qPCR reaction. It can obtain a good standard curve in a wide quantitative range, and accurately quantify and detect target genes with good repeatability and high reliability.

This kit contains special ROX, Passive Reference Dye, which is suitable for all qPCR instruments. There is no need to adjust the concentration of ROX on different instruments, and the amplification can be performed with the addition of primers and templates when preparing the reaction system.

Components

		2 × AceQ Universal SYBR qPCR Master Mix*
NB-54-0171-00	(100 rxns/20 µl reaction)	1.25 ml (sample size)
NB-54-0171-01	(500 rxns/20 µl reaction)	4 × 1.25 ml
NB-54-0171-02	(2,500 rxns/20 µl reaction)	5 x NB-54-0171-01

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

Storage

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

Applications

This reagent is used for the amplification and quantification of DNA samples, which can amplify DNA from all species, including genomic DNA, cDNA, plasmid DNA, λDNA, etc.

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 uses a special ROX, Passive Reference Dye, which is suitable for all qPCR instruments (no ROX calibration instrument, low concentration ROX calibration instrument, high concentration ROX calibration instrument), and there is no need to adjust the ROX concentration on different instruments.

Notes

1. This kit as far as possible to avoid repeated freezing and thawing to avoid the decrease of enzyme activity. If the amount of each use is small, it is recommended to repack into small aliquots.
2. Before use, please turn up and down to mix the Mix thoroughly. Do not vortex to avoid excessive bubbles and cause the reaction system volume to be inaccurate, which will affect the quantitative results. Mix can be used after mixing and centrifugation slightly. Pipette gently during use. If the Mix foams inadvertently, centrifuge it again before use.
3. This kit contains a fluorescent dye SYBR Green I, so it should be stored away from light. When preparing the reaction system, avoid strong light as far as possible.
4. Detection sensitivity of this kit is very high, easy to be contaminated by aerosols and lead to experimental failure. Therefore, the preparation of the reaction system should be carried out in the clean bench. Please use clean sterile tips and reaction tubes during the preparation process. In laboratories where conditions permit, special pipette and tips with filter element are recommended.

Experiment Process (Using ABI StepOnePlus)

1. Mix the following components thoroughly in qPCR tube

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

▲ In general, a better amplification effect can be obtained when the final concentration of primers is 0.2 µM. When the reaction performance is poor, the primer concentration can be adjusted in the range of 0.1 - 1.0 µM.

▲ qPCR is highly sensitive, and the accuracy of the amount of template added to the reaction system will have a great impact on the final quantitative results. Therefore, it is recommended that you dilute the template and add it to the reaction system, which can effectively improve the reproducibility of the experiment.

▲If the template type is undiluted crude liquid of cDNA, the volume used should not exceed 1/10 of the total volume of the qPCR reaction.

2. Perform qPCR reaction according to the following conditions

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

a. The AceTaq DNA Polymerase is a hot-start Taq DNA polymerase, so that the initial denaturation stage should be set at 95°C for at least 5 min, and it can be extended to 10 min if GC content of the template is high.
b. The extension time should be adjusted according to the minimum time required for data collection by the Real-time PCR instrument used. For ABI 7700 and 7900HT, the extension time should be ≥30 sec; for ABI 7000 and 7300, should be ≥31 sec; for ABI 7500, should be ≥34 sec; and for ABI StepOnePlus, should be ≥10 sec.
c. Different qPCR instrument types of melting curve acquisition procedures are not the same, and please select the instrument default melting curve acquisition procedures.

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 concentration of templates is too high and the baseline endpoint values are greater 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. Centrifuge carefully when handling the sample, and carefully check whether there are bubbles residue in the reaction tube before perform the amplification reaction.

No amplification plot

1. Insufficient cycling: Generally, the number of cycles is set to 40. However, it should be noted that too many cycles will increase too many background signals and reduce the reliability of data.
2. Confirm whether the signal acquisition step is set 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°C extension stage.
3. Confirm whether the primers are degraded: The integrity of primers that have not been used for a long time should be detected by PAGE electrophoresis to exclude 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 the template again and retry.

CT value appears too late

1. Low amplification efficiency: Optimize the PCR system. Try three-step program or re-design 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. Degradation of templates: Prepare new templates 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 folds or prepare new templates and retry.

Amplification observed in negative control

1. Contamination of reaction system: Replace with new mix, ddH2O and primers to repeat the experiment. The reaction system should be prepared in clean bench to reduce aerosol contamination.
2. Appearance of primer dimer: Carry out analysis in association with the melting curve.

The linear relationship of the standard curve is not satisfactory in absolute qualification

1. Deviations of pipetting volume: Increase the dilution ratio of template and increase the pipetting volume accordingly.
2. Degradation of standards: Prepare new standards and retry.
3. High template concentration: Increase the dilution fold.

Multiple peaks in melting curve

1. Unoptimized primers: Design and synthesize new primers according to the primer design principles.
2. Too high concentration of primers: Appropriately decrease the concentration of primers.
3. cDNA template with contamination of genomic DNA: Prepare new cDNA templates.

Experiment has low reproducibility

1. Inaccurate pipetting volume: Use higher performance pipette; increase the template dilution factor, and increase the sample loading volume.
2. Difference in temperature control between holes in qPCR instrument: Regularly calibrate the instruments.
3. Low template concentration: The lower the template concentration, the worse the reproducibility. Decrease the dilution fold or increase the volume of template addition.