ChamQ Universal SYBR qPCR Master Mix

Q711　　　Version 22.1

Product Description

ChamQ Universal SYBR qPCR Master Mix is a special premix for qPCR reaction using SYBR Green I chimeric fluorescence method. The core component, Champagne Taq DNA Polymerase, is a novel hot-start DNA polymerase modified by antibody method. It has many advantages such as strong specificity, high detection sensitivity. With the optimal Buffer optimized for qPCR and the specific promoter, it is very suitable for qPCR reaction with high specificity and sensitivity. 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

Components	Q711-02 (500 rxns/20 μl reaction)	Q711-03 (2,500 rxns/20 μl reaction)
2 × ChamQ Universal SYBR qPCR Master Mix*	4 × 1.25 ml	5 × Q711-02

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

Storage

Store at -30 ~ -15℃ and protect from light. Master mix can be stored stably at 2 ~ 8℃ for 6 months and protected from light after thawing. Transport at ≤ 0℃.

▲After the master mix is thawed, a little white precipitation may appear. Please place it at room temperature for a while and turn it up and down to dissolve the precipitate. Please confirm that the precipitation is completely dissolved and fully mixed before use.

Applications

This kit 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.

Notes

1. If white precipitate is found in the Master Mix after thawing, please place it at room temperature for a short while and turn it up and down to dissolve the precipitate before use.

2. 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 it to small portions.

3. Please turn the Master Mix upside down to mix thoroughly. Do not vortex to avoid air bubbles, which will affect the quantitative results. The Master Mix is ready to use after mixing and centrifuging briefly. Mix gently by pipetting. If the air bubbles are generated, please centrifuge again before use.

4. As this kit contains a fluorescent dye SYBR Green I, it should be stored protect from light. Avoid strong light when preparing the reaction solution.

5. Detection sensitivity of this kit is very high, easy to be contaminated by aerosols in the air. Therefore, the preparation of the reaction system should be carried out in clean bench. Sterile tips and reaction tubes should be used in the preparation process. If laboratory conditions permit, special pipettes and tips with filtering element are recommended.

Experiment Process

1. Prepare the following mixture in a qPCR tube

2 × ChamQ 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
ddH₂O	To 20.0 μl

The amount of each component in the reaction system 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 extremely sensitive, so the accuracy of the amount of template added to the reaction system will greatly affect the final quantitative results. It is recommended to 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]	Rep: 1	95℃	30 sec
Stage 2	Cycling Reaction	Reps: 40	95℃	3 - 10 sec[b]
			60℃	10 - 30 sec[c]
Stage 3	Melting Curve[d]	Rep: 1	95℃	15 sec
			60℃	60 sec
			95℃	15 sec

a. Initial denaturation condition is suitable for most amplification reactions. If the template structure is complex, the initial denaturation time can be extended to 3 min to improve the initial denaturation effect.

b. For standard program, select 10 sec, and 3 sec can be selected for fast program.

c. Select 30 sec for standard program; Fast program: for amplicons within 200 bp, the shortest extension time can be set to 10 sec; for amplicons over 200 bp, the recommended extension time is 30 sec.

d. 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 template concentration is too high and the baseline endpoint value 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: 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℃ extension stage.

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.

⑤ Template degradation: Prepare new template and retry.

◇ C_T value appears too late

① Low amplification efficiency: Optimize the PCR system, then try the three-step amplification program or redesign and synthesize 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.

◇ The linear relationship of standard curve is not satisfactory in absolute quantification

① Deviations of pipetting volume: Increase the dilution factor of template and increase the pipetting volume accordingly.

② Degradation of standards: Prepare new standards and retry.

③ High template concentration: Increase the dilution factor.

◇ Multiple peaks in the 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.