ChamQ SYBR qPCR Master Mix

Q311

Version 22.1

Product Description

ChamQ SYBR qPCR Master Mix is a special premix for qPCR assays based on SYBR Green I fluorescence method. The core component, Champagne Taq DNA Polymerase, is a novel antibody-modified hot-start DNA polymerase with high specificity and detection sensitivity. Equipped with the optimized buffer for qPCR and specificity-promoting factors, it is very suitable for highly specific and sensitive qPCR assays. This product is a 2 × premix reagent that contains SYBR Green I at an optimal concentration for qPCR. It can obtain a good standard curve in a wide quantitative range, and accurately quantify target genes with good repeatability and high reliability.

Components

Components	Q311-02 500 rxns (20 μl/rxn)	Q311-03 2,500 rxns (20 μl/rxn)
2 × ChamQ SYBR qPCR Master Mix[a]	4 × 1.25 ml	
50 × ROX Reference Dye 1[b]	200 μl	5 × Q311-02
50 × ROX Reference Dye 2[b]	200 μl	

a. It contains dNTP, Mg^{2+}, Champagne Taq DNA Polymerase and SYBR Green I, etc.

b. Used to correct the error of fluorescence signals between well. The recommended ROX Reference Dyes for various instruments are as follow:

No ROX Reference Dye	Bio-Rad CFX96, CFX384, iCycler iQ, iQ5, 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.
Add ROX Reference Dye 1 (1 ×)	Applied Biosystems 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast; StepOne, StepOnePlus.
Add ROX Reference Dye 2 (1 ×)	Applied Biosystems 7500, 7500 Fast, ViiA7; Stratagene MX4000, MX3005P, MX3000P.

Storage

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

▲ If white precipitate is found in the Master Mix after thawing, place it at room temperature for a short while and invert the tube upside down several times to dissolve the precipitate. Please make sure the precipitates have fully dissolved and mix thoroughly before use.

Applications

It is applicable for the amplification and quantification of DNA samples from all species, including genomic DNA, cDNA, plasmid DNA and λDNA.

Notes

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

1. If white precipitate is found in the Master Mix after thawing, please place it at room temperature for a short while and invert the tube upside down several times 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 Master Mix into small portions.
3. 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.
4. 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.
5. 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:

2 × ChamQ SYBR qPCR Master Mix	10.0 μl
Primer 1 (10 μM)	0.4 μl
Primer 2 (10 μM)	0.4 μl
50 × ROX Reference Dye 1	0.4 μl
Template DNA/cDNA	x μl
ddH_2O	To 20.0 μl

The amount 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 the total volume of qPCR system.

2. Run the qPCR program as follows:

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	Melt Curved[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. The melting curve acquisition programs of different qPCR instrument types are not the same. Please select the default melting curve acquisition program of the instrument.

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℃ 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.

⑤ Template degradation: Prepare new template and retry.

◇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.