ChamQ SYBR Color qPCR Master Mix (Without ROX)

NB-54-0245

Sommaire

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#Cat: NB-54-0245 Size: 500rxns

01/Product Description

ChamQ SYBR Color qPCR Master Mix (Without ROX) is a special 2 × 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 obtaina good standard curve in a wide quantitative range, and accurately quantify target genes withgood repeatability and high reliability. In addition, this product can track the pipetting process byusing the color change effect produced by the mixing of different dyes, there by significantly reducing pipetting errors.

02/Components

Components	NB-54-0245 500 rxns (20 µl/rxn)
2 × ChamQ SYBR Color qPCR Master Mix (Without ROX)[a]	4 × 1.25 ml
10 × Dilution Buffer[b]	1.25 ml

a. It contains dNTP, Mg2+, Champagne Taq DNA Polymerase, SYBR Green I, blue chromogenic dye, etc.
b. 10 × yellow concentrated template diluent.

03/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.

04/Applications

It is applicable for amplification reaction of animal, plant and microbial DNA.

05/Applicable Instruments

This product is not premixed with ROX Reference Dye to correct the fluorescence signal error between wells. It is suitable for the following qPCR instruments:

Bio-Rad CFX96, CFX384, iCycler iQ, iQ5, MyiQ; MiniOpticon, Opticon, Opticon 2, Chromo 4; Cepheid Smart-Cycler; 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 Cy-cler; and other fluorescence quantitative PCR instruments without the addition of ROX Reference Dye.

06/Notes

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

ChamQ SYBR Color qPCR Master Mix contains blue dye. 10 × Dilution Buffer contains yellow dye. When the amplification template (yellow) diluted with Dilution Buffer is added to ChamQ SYBR Color qPCR Master Mix (blue), it will produce the color change effect (blue→ green), so that you can accurately determine whether the template has been added according to the color of the liquid.

1. 10 × Dilution Buffer is a special concentrated template diluent buffer. During use, if pipetting tracking is required, select the appropriate method to add Dilution Buffer to the template in advance according to the following table, and then perform qPCR detection; if pipetting tracking is not required, do not use Dilution Buffer.

Template status	**Examples**	**10 x Dilution Buffer usage**	**Dilution Buffer concentration in the template**
Plaque/Powder	Undisolved DNA preciptated plaques	Use ddH2O to dilute 10 × Dilution Buffer to 1 ×, and then use an appropriate volume of 1 × Dilution Buffer to dissolve plaque/powder	1 x
Solution	cDNA solution, dissolved plasmid, genome	If necessary, dilute the template to the target concentration with ddH2O, and then add 1 µl 10 × Diffusion Buffer to each 9 µl template.	1 x

▲ Other addition schemes can also be used in actual use. The general principle of use is that the concentration of Dilution Buffer in the final template is 1 ×.

2. If Dilution Buffer is used for pipetting tracking (qPCR template includes 1 × Dilution Buffer), the volume of the template should not exceed the range of 2 - 5 µl/20 µl reaction. If the template usage is less than 2 µl/20 µl reaction, the color will be lighter and affect the tracking effect; if the template usage is higher than 5 µl/20 µl reaction, the active component in the Dilution Buffer may interfere with the qPCR assay.
3. If the Dilution Buffer is used improperly, it may have a certain impact on the qPCR results.
4. This product comes with a white loaded plate. When mixing with transparent tubes/plates, place the loaded plate under the transparent tubes/plates to significantly improve the color contrast and make pipetting tracking easier.

07/ Experiment Process (Using Bio-Rad CFX96)

1. Mix the following components thoroughly in qPCR tube:

2 × ChamQ SYBR Color qPCR Master Mix (Without ROX)	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 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.

▲ Special reminder 1: When the template already contains 1 × Dilution Buffer, the addition volume of template should not exceed the range of 2 - 5 µl/20 µl reaction. The larger the volume of the template used in the recommended range, the more obvious the color change will be.

▲ Special reminder 2: The volume of undiluted cDNA template (regardless of whether it contains 1 × Dilution Buffer) 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[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. 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 amplicons within 300 bp, set the extension time to 30 sec; for amplicons over 300 bp, it is recommended to increase the extension time to 60 sec.

c. 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.

08/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 CT value. Reduce the baseline endpoint (CT 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.

◇ **CT 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

◇ **Amplification observed in negative control**

① Contaminated 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 Primer dimer: Carry out analysis in association with the melting curve.

◇ **Multiple peaks in melting curve**

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

② principles. 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.