CYPC33-2 x SYBR Green qPCR Mix

Contents	50 rxns (CYPC3301)	200 rxns (CYPC3302)
2 x SYBR qPCR Mix	1.25 ml (50ul reaction system ×50 reactions)	4×1.25ml (50ul reaction system ×200 reactions)

Storage and Stability

2 x SYBR Green qPCR Mix should be stored protected from light at -20°C for long term storage (more than 6 months), or at 4°C for short term storage. Avoid repeated freezing and thawing.

Introduction

2 x SYBR Green qPCR Mix provides rapid real-time quantification of RNA targets in SYBR Green fluorescent. The components of 2 x SYBR Green qPCR Mix include Hot-start enzyme named HotMaster Taq DNA Polymerase, dNTP, PCR reaction buffer, BSA and SYBR Green, and so on. The fluorescent dye SYBR Green I in the master mix enables the analysis of many different targets without having to synthesize target specific labeled probes. High specificity and sensitivity in PCR are achieved by the use of the hot-start enzyme HotMaster Taq DNA Polymerase together with a specialized PCR buffer.

Unlike other “hot-start” Taq DNA polymerase formulations that block the enzyme activity only in the first high temperature step, HotMaster Taq DNA inhibitors closes the polymerase-substrate binding site by temperature adjustment method. Inactive polymerase-inhibitor complexes are formed at temperatures below 40°C. As the temperature is elevated to the primer-specific annealing temperature, the binding equilibrium is shifted towards complex formation only with target-specific primed template DNA. This prevents the formation of mis-primed products and primer–dimers during reaction setup and the first denaturation step, leading to high PCR specificity and accurate quantification.

Note:

1. Reference Dye ROX is not included in 2 x SYBR Green qPCR Mix. It is for the researcher to decide whether or not ROX reference dye should be included in the experiment, on the basis of technical protocol of qPCR instruments. Its aim is to eliminate the background signal and to adjust and compensate lot-to-lot fluorescence signal error. The catalog number of the supporting ROX product is PC38 ROX reference dye.
2. SYBR® Green I dye is light sensitive and should be stored in the dark, and be protected from light when use.
3. We strongly recommend preparing the PCR reaction on ice, for higher specificity and

less background.

4. Mg2+ concentration in 2 x SYBR Green qPCR Mix is 4 mM. We strongly recommend an initial Mg2+ concentration of 2mM. Proper Mg2+ concentration can be achieved by using 25mM MgCl2.

Reaction setup: We recommend assembling all reaction components on ice.

Components	Volume（25 μl）	Volume（50 μl）	Final Concentration
2 x SYBR qPCR Mix	12.5 μl	25 μl	1 x
DNA Template	1 μl	2 μl	as required
Forward Primer (10 μM)	0.5 μl	1 μl	0.2 μM each
Reverse Primer (10 μM)	0.5 μl	1 μl	0.2 μM each
ddH2O to final volume	25 μl	50 μl	Not applicable

Real-time cycler conditions (Two-Step)

94C° 2-3 min

94C°5-10 sec

60C° 30-34 sec 40 cycles

Dissociation Stage

Real-time cycler conditions (Three-Step)

94C° 2-3 min

94C°10-20 sec

55-60C° 10-20 sec 40 cycles

72C° 20-30 sec

Dissociation Stage

The Mix is compatible with all real-time PCR instruments. In most cases it can get good results using Two-Step or Three-Step method. The program can be fine-tuned according to the instruments in use. In general, Two-Step has a higher specificity, while Three-Step has higher efficiency. If the melting curve is poor, a two-step method is recommended. If the Tm value of primers is too lower, increasing extension time or three-step method can solve.

Troubleshooting Guide

A1: No product, or product detected late in PCR, or only primer–dimers detected

Q1:

1. Insufficient number of cycles. Increase the number of cycles.
2. Wrong detection step. Ensure that fluorescence detection takes place during the extension step of the cycling program.
3. Primers degraded. Check for possible degradation of primers on a denaturing polyacrylamide gel.
4. Primer design not optimal. Check for PCR products by melting curve analysis or gel electrophoresis.
5. Insufficient amount of starting template. Increase the amount of template, if possible. Ensure that sufficient copies of the target nucleic acids are present in your sample

A2: No linearity in ratio of CT value/crossing point to log of the template amount

Q2:

1. Template amount too high. Do not exceed the maximum recommended amount of template.
2. Template amount too low. Increase the amount of template, if possible.

PCR product too long. For optimal results, PCR products should be between 100 and 150 bp. PCR products should not exceed 500 bp.

Typical Data: Amplification curve and Melting Curve

Using 2 x SYBR Green qPCR Mix in Roche Light Cycler 480 II with Three-Steps method