SYBR Color qPCR SuperMix

Cat #: orb1736807 (manual)

For research use only. Not intended for diagnostic use.

Product Features

Specification: 20 µL x 500 rxns

Storage: -20℃ (protected from light)/ 1 year.

Application: The SYBR Color qPCR SuperMix can be used for gene expression analysis of cDNA, absolute quantification of plasmids, gDNA and sequencing libraries.

Introduction:

SYBR Color qPCR SuperMix is an optimized 2× real-time PCR premix containing HotStarTaq DNA polymerase, SYBR Green I fluorescent dye, dNTPs, Mg^{2+}, and High ROX. The balanced K^+ and NH_4^+ ion ratios in the buffer promote specific primer annealing, ensuring high sensitivity and specificity. The PCR reaction can be initiated simply by adding primers and cDNA template to the ready-to-use master mix. The unique buffer formulation enables sensitive qPCR performance on all real-time PCR instruments without the need for optimization.

In addition, the product allows visualization of the sample addition process through a color change after the template is added, which greatly improves pipetting efficiency and helps prevent spiking errors.

Principle

SYBR Color qPCR SuperMix enables specific and sensitive detection across a wide dynamic range on both standard and rapid PCR instruments. The SYBR Green I dye included in the premix allows analysis of multiple target nucleic acids without the need for synthetic sequence-specific probes. The specially formulated Rapid PCR buffer significantly reduces denaturation, annealing, and extension times and is well suited for complex templates, samples containing high levels of PCR inhibitors (such as soil or fecal DNA), and long-fragment amplification. In addition, HotStarTaq DNA Polymerase is activated by heating at 95 °C for 30 seconds and requires a strict hot-start procedure to prevent the generation of non-specific products.

Components

Component	500 rxns
2 x SYBR Color qPCR Mix	4 x 1.25 ml
10 x Dilution Buffer	1 ml
50 x ROX Dye	1 ml
ddH2O	4 x 1.25 ml
User manual	1 copy

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27713. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558

10 x Dilution Buffer

2 x SYBR Color qPCR SuperMix is supplemented with blue dye, and 10 x Dilution Buffer contains yellow dye. When SYBR Color qPCR SuperMix (blue) is added with amplification template (yellow) diluted with Dilution Buffer, a blue → green color change reaction is generated, so that it is possible to accurately determine whether template has been added based on the liquid color.

1. 10 x Dilution Buffer is a special concentrated template dilution solution. For pipetting traces during the experiment, add 10 x Dilution Buffer to the diluted template solution (e.g. cDNA, plasmid, gDNA solution, etc.) so that the final concentration of Dilution Buffer in the template is 1 x. Example: Dilute the template to the target concentration using ddH2O, then add 1 µL of Dilution Buffer to every 9 µL of template dilution solution.

2. When using Dilution Buffer for pipette tracking, the amount of template added is 2 µL/20 µL reaction. low additional amount may lead to light color development and affect the tracking effect; too high amount may interfere with the qPCR reaction.

3. Dilution Buffer is not used if pipetting tracking is not required.

Template

cDNA: For two-step quantitative qPCR, use 10 µL of cDNA reverse transcribed from 10 pg to 1 ng of total RNA.

In the 20 µL reaction system, the amount of cDNA template used is generally not more than 100 ng. It should be noted that when detecting high-abundance genes in undiluted cDNA, the Ct value in quantitative PCR results may be too low, which may affect the accuracy of quantification. Gradient dilution of the cDNA template results in more accurate results.

Plasmid and genomic DNA: 100 pg to 1 ng of genomic DNA or 101 -107 copies of plasmid DNA can be used in a 20 µL system.

ROX Dyes

The fluorescence signal in the reaction system can be normalized by adding ROX dye to the reaction system according to the instrument of choice. The table below shows the amount of ROX (per 20 µL reaction system) required for operation with different instruments.

Instrument	Amount of ROX required per 20 µL system reaction
ABI7300, 7900HT, StepOne, etc.	2 µL
ABI7500, 7500Fast, ViiA7, Stratagene Mx3000™, Mx3005P™, and Mx4000™, etc.	0.4 µL
Roche, Bio-Rad, Eppendorf, etc.	No need to add

Reaction System

A reaction system as described below was established. To perform multiple reactions, prepare a premix of the common components, add a suitable volume to each tube or well, and then add a special reaction component (eg, template).

Composition	Dosage
2 x SYBR Color qPCR Mix	10 µL
PCR Forward Primer (10 µM)	0.4 µL
PCR Reverse Primer (10 µM)	0.4 µL
Template DNA/cDNA*	x µL
*50 x ROX Dye (optional)	0.4 µL
ddH2O	up to 20 µL

***Recommended addition of diluted templates**

1. It is recommended to use a 20µL system to ensure the validity and repeatability of the amplification of the gene of interest.

2. Cover or seal the reaction tube/PCR plate and mix gently. It can be centrifuged slightly to ensure that all components are at the bottom of the tube.

3. Place the reaction system in a real-time PCR instrument, collect data and analyze the results. Set up your PCR instrument as shown in the table below. Optimum temperature and the incubation time can be determined by the specific situation.

Two-step amplification procedure

Stage	Number of cycles	Temperature	Time
Pre-denaturation	1x	95°C	30 sec
Denaturation	35-40x	95°C	5 sec
Annealing/extension		60°C	30 sec
Melt Curve			

Three-step amplification procedure

Stage	Number of cycles	Temperature	Time
Pre-denaturation	1x	95℃	30 sec
Denaturation	35-40x	95℃	5 sec
Annealing		50-60℃	30 sec
extension		72℃	30 sec
Melt Curve			

Note:

1. Pre-denaturation time: Satisfy with the amplification of most genes. If the amplified fragment is a fragment with high GC content or a complex structure sample, the pre-denaturation time can be increased to 2 min.

2. Annealing temperature and time: can be adjusted according to the primer Tm value and target gene amplification length.

3. Melting curve: The default program of the instrument is usually used.

Calculation of Results

Quantitative experiments require at least three biological replicates. After the reaction is completed, it is necessary to confirm the amplification curve and the melting curve.

Declaration

1. For research use only. Not intended for diagnostic use.

2. Store at -20℃ in the dark. This product contains the fluorescent dye SYBR Green. When storing or formulating the reaction system, avoid strong light. Please mix it upside down before using it.

Safety Notes

For your safety and health, please wear a lab coat and wear disposable gloves when performing the experiment.

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27713. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558