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EnTurbo™ SYBR Color qPCR SuperMix

Catalog No.	Specification	Storage/Shelf life
EQ036-1	20 μL x 500 rxns	-20℃/one year

Advantage

1) Quickly get results, saving up to 50% of the time
2) Optimized ready-to-use master premix for rapid PCR reactions
3) Accurate detection of various starting amounts of template, stable amplification quantitative results with high repeatability
4) Balanced K^+ and NH_4^+ ion ratios ensure high sensitivity and specificity
5) Stand-alone ROX Reference Dye package for all Real-time PCR instruments
6) Reduces pipetting errors by tracking the pipetting process through the color change reaction between dyes

Introduction

EnTurbo™+ SYBR Color qPCR SuperMix is an optimized 2 x Real-time PCR premix containing HotStarTaq DNA Polymerase, SYBR Green I fluorochrome dye, dNTP and Mg2+ and High ROX. In addition, the balanced K+ and NH4+ ion ratios in the buffer promote specific primer annealing, to ensure a highly sensitive and specific PCR reaction, the reaction can be initiated by simply adding the primer and cDNA template to the ready-to-use PCR master premix. The unique PCR buffer ensures sensitive qPCR on all real-time PCR instruments without optimization.

In addition, the product visualizes the spiking process through the color change reaction after the template is added, which greatly improves the spiking efficiency and avoids spiking errors.

*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.

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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

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
ddH_2 O	4 x 1.25 ml
User manual	1 copy

Principle

The EnTurbo™ SYBR Color qPCR SuperMix allows for specific, sensitive detection over a wide range for both standard and rapid PCR instruments. The SYBR Green I dye in the premix allows analysis of multiple target nucleic acids without the need for synthetic sequence-specific probes. The specially formulated Rapid PCR buffer greatly reduces denaturation, annealing and extension times and is well suited for complex templates, templates with high PCR inhibitor residues (e.g. soil and fecal DNA) and long fragment amplification. In addition, HotStarTaq DNA Polymerase can be activated by heating at 95°C for 30 sec and requires a strict hot start to avoid generation of non-specific products.

Application

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

Attention

1. Transportation and storage

1) Ice bag ,dry ice transport.

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 use.

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

2. 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 10^1 -10^7 copies of plasmid DNA can be used in a 20 μL system.

3. 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 ddH_2O, then add 1 μL of Dilution Buffer to every 9 μL of template dilution solution. 10 x Dilution Buffer.

2) When using Dilution Buffer for pipette tracking, the amount of template added is 2 μL/20 μL reaction. low addition 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.

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
ddH_2O	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℃	30 sec
Denaturation	35-40x	95℃	5 sec
Annealing/extension		60℃	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 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.

Result analysis

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.