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EnTurbo™ SYBR Color qPCR SuperMix (Low ROX Premixed)

Catalog No.	Specification	Storage/Shelf life
EQ033-1	20 μL x 500 rxns	-20℃/ one year

Adavantage

1) Quickly get results, saving up to 50% of the time
2) Optimized ready-to-use pre mix for rapid PCR reactions
3) Accurate detection of various starting amounts of templates, stable amplification, quantitative results with high repeatability
4) Balanced K^+ and NH_4^+ ion ratios,ensure high sensitivity and high specificity
5) Track the pipetting process and reduce pipetting errors through the color change reaction between dyes.

Applicable Type

ABI7500, 7500FAST, ViiA7, Stratagene Mx3000™, Mx3005P™, Mx4000™.

Introduction

EnTurbo™ SYBR Color qPCR SuperMix is an optimized 2x real-time PCR master mix containing HotStarTaq DNA Polymerase, SYBR Green® fluorescent dye, dNTP and Mg2+, low 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 mix. Greatly simplify the operation process and reduce the probability of pollution.The unique PCR buffer ensures sensitive qPCR on all Real-Time PCR instruments without optimization.

In addition, the product realizes the visualization of the sample addition process through the discoloration reaction after the template is added, which greatly improves the sample addition efficiency and avoids sample addition errors.

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Components

Component	500 rxns
2 x SYBR Color qPCR Mix (Low ROX)	4 x 1.25 ml
10 x Dilution Buffer	1 ml
ddH_2O	4 x 1.25 ml
User manual	1 copy

Principle

EnTurbo™ SYBR Color qPCR SuperMix provides a wide range of specific, sensitive assays for standard and rapid PCR machines. SYBR Green® fluorescent dye in the master mix can analyze multiple target nucleic acids without the need to synthesize sequence-specific probes. The special fast PCR buffer can greatly shorten the denaturation, annealing and extension time, and has good applicability to complex templates, templates with more PCR inhibitor residues (such as soil and fecal DNA) and long fragment amplification. In addition, HotStarTaq DNA Polymerase can be activated by heating at 95°C for 30 sec, requiring a strict hot start to avoid nonspecific products.

Application

EnTurbo™ SYBR Color qPCR SuperMix can be used for cDNA gene expression analysis, absolute quantification of plasmids, gDNA and sequencing libraries for a variety of Real-Time PCR instruments.It is suitable for various real-time PCR machines, including PCR machines from ABI, Bio-Rad, Eppendorf, Roche and Agilent.

Attention

1. Transportation and storage

1) Ice bag ,dry ice transport.

2) Store at -20℃ in the dark. This product contains SYBR Green® fluorescent dye. 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 10μL of cDNA reverse transcribed from total RNA (10pg to 1ng).

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: 100pg to 1ng of genomic DNA or 10^1-10^7 copies of plasmid DNA can be used in a 20μL system.

3. 10 x Dilution Buffer

2 x SYBR Color qPCR SuperMix containing blue dye， 10 x Dilution Buffer containing yellow dye. When the amplification template (yellow) diluted with Dilution Buffer is added to the SYBR Color qPCR SuperMix (blue), a blue-to-green color-changing reaction will occur, so that the color of the liquid can accurately determine whether the template has been added.

1） 10 x Dilution Buffer is a Specialized Concentrated Template Diluent. If pipetting trace is required during the experiment, add 10 x Dilution Buffer to the diluted template solution (e.g. cDNA, plasmids, gDNA solution, etc.)， make the concentration of Dilution Buffer in the final template 1x. Example: Dilute the template to the target concentration using ddH_2O, then add 1 μL of 10 x Dilution Buffer to each 9 μL of template dilution.

2） When using Dilution Buffer for pipetting tracking, template addition is 2 μL/20 μL reaction. Low addition may lead to light color development and affect the tracing effect; too high usage 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 (Low ROX)	10 μL
PCR Forward Primer (10 μM)	0.4 μL
PCR Reverse Primer (10 μM)	0.4 μL
Template DNA/cDNA*	x μL
ddH_2O	up to 20μL

*Suggested to add diluted templates.

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

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. 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	35-40 x	95℃	5 sec
Annealing		50~60℃	30 sec
Extension		72℃	30 sec
Melt Curve			

Note

1）Pre-denaturation time: To meet the amplification of most genes, if the amplified fragments are high GC content fragments or complex structural samples, the pre-denaturation time can be increased to 2 min.

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

3）Melting curve: Usually the instrument default program is 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.