EnTurbo™ SYBR Green PCR SuperMix(Low ROX Premixed)

Catalog No.	Specification	EnTurbo™ SYBR Green PCR SuperMix(Low ROX Premixed)	RNase-Free ddH2O	Storage/Shelf life
EQ014	20μL×500 rxns	4 x 1.25mL	4 x 1.25mL	-20℃/one year

Advantage

1) Quickly get results, saving up to 50% of the time
2) Optimized ready-to-use master 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 NH4+ ion ratios, ensure high sensitivity and high specificity.

Applicable Type

ABI7500、7500Fast、 ViiA7、Stratagene Mx3000™、Mx3005P™ and Mx4000™ etc.

Introduction

EnTurbo ™ SYBR Green PCR SuperMix is an optimized 2x real-time PCR master mix containing HotStarTaq DNA Polymerase, SYBR Green® fluorescent dye, dNTP ,Mg2+ and LowRox. 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. The unique PCR buffer ensures sensitive qPCR on all real-time PCR instruments without optimization.

Kit Components

Component	Character	Advantage
HotStarTaq DNA Polymerase	Heating at the pre-denaturation temperature for 30s will completely inactivate the blocking antibody and release the DNA polymerase activity	Effectively suppresses non-specific amplification caused by primer annealing
SYBR Green qPCR Buffer	Suitable for all real-time PCR instruments	qPCR run time is reduced by 50%, results are obtained faster, and more PCR reactions can be completed in one day
SYBR Green I dye	Strong fluorescence signal when combined with DNA duplex	High sensitivity amplification. It provides a wide-area linear range with a Ct value of 5-35 and a high sensitivity for single-digit copy detection, and is suitable for melting curve analysis.

Kit principle

EnTurbo™ SYBR Green PCR SuperMix provides a wide range of specific, sensitive assays for standard and rapid PCR machines. The SYBR Green I 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.

Kit application

EnTurbo™ SYBR Green PCR 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.

Attention

1. 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 10-107 copy number of plasmid DNA.

can be used in a 20μL system

2. Transportation and storage

1) Ice bag or dry ice bag transport.

2) Store at 2-8 °C in the dark. This product contains the fluorescent dye SYBR® Green I. 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.

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	96 wells		384 wells	Final Concentration
	50μL reaction system	20μL reaction system	10μL reaction system	——
2 x SYBR Green PCR Master Mix (Low ROX Premixed)	25μL	10μL	5μL	1 x
PCR Forward Primer (10 μM)	1μL	0.4μL	0.2μL	0.2μM
PCR Reverse Primer (10 μM)	1μL	0.4μL	0.2μL	0.2μM
Template				
RNase-Free ddH2O	to 50μL	to 20μL	to 10μL	——

1. It is recommended to use a 20μL or 50μL 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

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
Melting Curve (Melting 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
Melting curve(Melting Curve)			

Note:

1) Pre-denaturation time: to meet the amplification of most genes, if the amplified fragment is a high GC content fragment or a complex structure sample, the pre-denaturation time can be increased to 2-5min.

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 use the default program of the instrument.

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.