ELK Biotechnology

For research use only.

EnTurbo™ SYBRGreen PCR SuperMix

Catalog No.	Specification	EnTurbo™ SYBR Green PCR SuperMix	50x ROX Dye	RNase-Free ddH_2O	Storage/Shelf life
EQ001	20μL×500 rxns	4 x 1.25mL	1mL	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 NH_4^+ ion ratios, as well as stand-alone ROX Reference Dye packaging for all real-time PCR instruments

Introduction

EnTurbo™ SYBR Green PCR SuperMix is an optimized 2x real-time PCR master mix containing HotStarTaq DNA Polymerase, SYBR Green® fluorescent dye, dNTP and Mg^{2+}. In addition, the balanced K^+ and NH_4^+ 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

ELK Biotechnology

For research use only.

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.
ROX dye	Calibration of fluorescent signals on ABI and Agilent PCR machines	Calibration of PCR machines that require ROX dyes does not affect PCR reaction results

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-range amplification. In addition,HotStarTaq DNA Polymerase can be activated by heating at 95℃ for 30sec,requiring a strict hot start to avoid nonspecific products.

ELK Biotechnology

For research use only.

Kit application

EnTurbo™ SYBR Green PCR SuperMix Can be used for gene expression analysis of cDNA, plasmids, gDNA,absolute quantitative analysis. It is suitable for various real-time PCR machines, including ABI, Bio-Rad, Eppendorf, Roche and Agilent PCR machines.

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

2. Transportation and storage

1) Ice bag, dry ice transportation.

2) Store at -20℃ 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	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				
*50 x ROX Dye(Optional)	1μL	0.4μL	0.2μL	1x
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.

ROX dye

The fluorescent signal in the reaction system can be standardized by adding a ROX dye to the reaction system according to the selected instrument. The table below lists the amount of ROX required per unit of operation (per 50μL of reaction system):

Instrument	The amount of ROX required for each 50 μL system reaction
ABI7300、7900HT、StepOne etc.	5μL
ABI7500、7500Fast、 ViiA7、Stratagene Mx3000™、Mx3005P™ and Mx4000™ etc.	1μL
Roche、Bio-Rad，Eppendorf etc.	No need to add

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 stage			

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 analysis(Melting Curve stage)			

Note: The annealing temperature and time can be adjusted according to the length of the primer and the gene of interest.

The pre-denaturation condition is usually set at 95°C for 30 sec. Using this condition, the circular plasmid DNA and genomic DNA template that are difficult to denature can basically be denatured well. If you want to change the denaturation conditions for difficult-to-denaturate templates, you can extend it to 1 to 2 minutes. However, the enzyme is prone to inactivation for too long, so denaturation conditions of more than 2 minutes are not recommended.

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.