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EnTurbo™ Probe qPCR SuperMix

Catalog No.	Specification	EnTurbo™ Probe qPCR SuperMix	50x ROX Dye	RNase-Free ddH2O	Storage/Shelf life
EQ017	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 NH4+ ion ratios, as well as stand-alone ROX Reference Dye packaging for all real-time PCR instruments

Introduction

EnTurbo ™ Probe qPCR SuperMix is an optimized 2x real-time PCR master mix containing HotStarTaq DNA Polymerase, dNTP and Mg2+. 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.

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Kit Components

Component	Character	Advantage
HotStarTaq DNA Polymerase	Activated at 95℃ for 3 minutes	Construction of qPCR reaction system at room temperature
Probe 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
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™ Probe qPCR SuperMix provides a wide range of specific, sensitive assays for standard and rapid PCR machines. 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 must be heated at 95°C for 3 minutes to activate, requiring a rigorous hot start to avoid the formation of non-specific products.

Kit application

EnTurbo ™ Probe 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, including ABI, Bio-Rad, Eppendorf, Roche and Agilent.

Attention

1. Template

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

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 can be used in a 20μL system Copy number of plasmid DNA.

2. Transportation and storage

1) Ice bag transport.

2) Store at 2-8 °C in the dark. 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	96wells		384 wells	Final Concentration
	50μL reaction system	20μL reaction system	10μL reaction system	——
2 x Probe qPCR 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
Probe (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

Note: The annealing temperature can be adjusted according to the Tm value of primers.

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.