NeoProbe qPCR Turbo Mix

#Cat: NB-88-00087-5ml Size: 5ml

#Cat: NB-88-00087-25ml Size: 25ml

Specification	NeoProbe qPCR Turbo Mix	50x ROX Dye	RNase-Free ddH2O	Storage/Shelf life
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

NeoProbe qPCR Turbo Mix is a 2x master mix designed for probe-based qPCR, 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.

Kit Components

Component	Character	Advantage
HotStarTaq DNA Polymerase	Heating at the pre-denaturation temperature for 30 s will completely inactivate the blocking antibody and release the DNA polymerase activity.	It can effectively suppress non-specific amplification caused by primer annealing.
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

NeoProbe qPCR Turbo Mix 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 can be activated by heating at 95°C for 30 sec, requiring a strict hot start to avoid nonspecific products

Kit application

NeoProbe qPCR Turbo Mix 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, 10 µL of cDNA reverse-transcribed from 10 pg to 1 ng of total RNA was used.

In the 20µL 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 and dry ice transport.

2)Store at 2-8 ° C in the dark. Store at -20 ° C for long-term storage. 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
	20μL reaction system	10μL reaction system	
2 x NeoProbe qPCR Turbo Mix	10μL	5μL	1 x
PCR Forward Primer (10 μM)	0.4μL	0.2μL	0.2μM
PCR Reverse Primer (10 μM)	0.4μL	0.2μL	0.2μM
Probe probe（10uM ）	0.4μL	0.2μL	0.2μM
Template			
*50 x ROX Dye (optional)	0.4μL	0.2μL	1x
RNase-free ddH2O	to 20μL	to 10μL	——

1. It is recommended to use 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 ensurethat 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. Setup your PCR instrument as shown in the table below. Optimum temperature
4. 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 RO 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

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

Note:

Pre-denaturation time: It is suitable for the amplification of most genes. If the amplified fragments are high GC content fragments or samples with complex structures, the pre-denaturationtime can be increased to 2-5min.

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

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.