



## **EnTurbo™ Probe qPCR SuperMix**

**Cat# NB-88-00087**

| <b>Catalog No.</b> | <b>Specification</b> | <b>EnTurbo™ Probe qPCR<br/>SuperMix</b> | <b>50x ROX Dye</b> | <b>RNase-Free<br/>ddH2O</b> | <b>Storage/Shelf life</b> |
|--------------------|----------------------|---|--------------------|-----------------------------|---------------------------|
| <b>NB-88-00087</b> | 20µL×500 rxns        | 4 x 1.25mL                              | 1mL                | 4 x 1.25mL                  | -20°C/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<sup>+</sup> and NH<sub>4</sub><sup>+</sup> ion ratios, as well as stand-alone ROX Reference Dye packaging for all real-time PCR instruments

### **Introduction**

EnTurbo™ Probe qPCR SuperMix is a 2x master mix designed for probe-based qPCR, containing HotStarTaq DNA Polymerase, dNTP and Mg<sup>2+</sup>. In addition, the balanced K<sup>+</sup> and NH<sub>4</sub><sup>+</sup> 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

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 can be activated by heating at 95°C for 30 sec, requiring a strict hot start to avoid nonspecific 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, 10  $\mu$ L of cDNA reverse-transcribed from 10 pg to 1 ng of total RNA was used.

In the 20 $\mu$ L 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 20 $\mu$ L 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 |
|---------------------------------|----------------------------|----------------------------|---------------------|
|                                 | 20 $\mu$ L reaction system | 10 $\mu$ L reaction system |                     |
| 2 x Probe qPCR SuperMix         | 10 $\mu$ L                 | 5 $\mu$ L                  | 1 x                 |
| PCR Forward Primer (10 $\mu$ M) | 0.4 $\mu$ L                | 0.2 $\mu$ L                | 0.2 $\mu$ M         |
| PCR Reverse Primer (10 $\mu$ M) | 0.4 $\mu$ L                | 0.2 $\mu$ L                | 0.2 $\mu$ M         |
| Probe probe (10 $\mu$ M)        | 0.4 $\mu$ L                | 0.2 $\mu$ L                | 0.2 $\mu$ M         |
| Template                        |                            |                            |                     |
| *50 x ROX Dye (optional)        | 0.4 $\mu$ L                | 0.2 $\mu$ L                | 1x                  |
| RNase-free ddH <sub>2</sub> O   | to 20 $\mu$ L              | to 10 $\mu$ L              | ---                 |

1. It is recommended to use 20 $\mu$ L 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 50 $\mu$ L 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°C        | 30 sec |
| Denaturation        | 35-40x           | 95°C        | 5 sec  |
| Annealing/extension |                  | 60°C        | 30 sec |

### Three-step amplification procedure:

| Stage            | Number of cycles | Temperature | Time   |
|------------------|------------------|-------------|--------|
| Pre-denaturation | 1x               | 95°C        | 30 sec |
| Denaturation     | 35-40x           | 95°C        | 5 sec  |
| Annealing        |                  | 50°C-60°C   | 30 sec |
| Extension        |                  | 72°C        | 30 sec |

### Note:

- 1) 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-denaturation time can be increased to 2-5min.
- 2) Annealing temperature and time: It can be adjusted according to the T<sub>m</sub> 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.