



**ELK Biotechnology**

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## EnTurbo™ SYBR Color qPCR SuperMix (Without ROX)

Catalog No.	Specification	Storage/Shelf life
EQ035-1	20 µL x 500 rxns	-20°C/ one year

### Advantage

- 1) Quickly get results, saving up to 50% of the time
- 2) Optimized ready-to-use pre 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, ensure high sensitivity and high specificity
- 5) Track the pipetting process and reduce pipetting errors through the color change reaction between dyes.

### Introduction

EnTurbo™ SYBR Color qPCR SuperMix is an optimized 2x real-time PCR master mix containing HotStarTaq DNA Polymerase, SYBR Green® fluorescent dye, 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. Greatly simplify the operation process and reduce the probability of pollution. The unique PCR buffer ensures sensitive qPCR on all Real-Time PCR instruments without optimization.

In addition, the product realizes the visualization of the sample addition process through the discoloration reaction after the template is added, which greatly improves the sample addition

### Applicable Type

This product does not contain ROX Reference Dye, which is used to correct the fluorescence signal error between wells, and is suitable for the following Real-Time PCR instruments: Roche instruments, Bio-Rad instruments, Eppendorf instruments, etc.; and other Real-Time PCR instruments that do not require the addition of ROX Reference Dye.



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### Kit Components

Component	500 rxns
2 x SYBR Color qPCR Mix (Without ROX)	4 x 1.25 ml
10 x Dilution Buffer	1 ml
ddH <sub>2</sub> O	4 x 1.25 ml
User manual	1 copy

### Kit principle

EnTurbo™ SYBR Color qPCR SuperMix provides a wide range of specific, sensitive assays for standard and rapid PCR machines. SYBR Green® fluorescent 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 Color 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. It is suitable for various real-time PCR machines, including PCR machines from ABI, Bio-Rad, Eppendorf, Roche and Agilent.

### Attention

#### 1. Transportation and storage

- 1) Ice bag ,dry ice transport.
- 2) Store at -20°C in the dark. This product contains SYBR Green® fluorescent dye. 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.

#### 2. 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



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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  $10^1$ - $10^7$  copies of plasmid DNA can be used in a 20µL system.

### 3. 10 x Dilution Buffer

2 x SYBR Color qPCR SuperMix containing blue dye, 10 x Dilution Buffer containing yellow dye. When the amplification template (yellow) diluted with Dilution Buffer is added to the SYBR Color qPCR SuperMix (blue), a blue-to-green color-changing reaction will occur, so that the color of the liquid can accurately determine whether the template has been added.

- 1) 10 x Dilution Buffer is a Specialized Concentrated Template Diluent. If pipetting trace is required during the experiment, add 10 x Dilution Buffer to the diluted template solution (e.g. cDNA, plasmids, gDNA solution, etc.), make the concentration of Dilution Buffer in the final template 1x. Example: Dilute the template to the target concentration using ddH<sub>2</sub>O, then add 1 µL of 10 x Dilution Buffer to each 9 µL of template dilution.
- 2) When using Dilution Buffer for pipetting tracking, template addition is 2 µL/20 µL reaction. Low addition may lead to light color development and affect the tracing effect; too high usage may interfere with the qPCR reaction.
- 3) Dilution Buffer is not used if pipetting tracking is not required.

### 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	Dosage
2 x SYBR Color qPCR Mix (Without ROX)	10 µL
PCR Forward Primer (10 µM)	0.4 µL
PCR Reverse Primer (10 µM)	0.4 µL
Template DNA/cDNA*	x µL
ddH <sub>2</sub> O	up to 20µL

\*Suggested to add diluted templates.

- 1) It is recommended to use a 20µL system to ensure the validity and repeatability of the amplification of the target gene.



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- 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. Optimum temperature and the incubation time can be determined by the specific situation.

### 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
Melt Curve			

### Three-step amplification procedure:

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

### Note

- 1) Pre-denaturation time: To meet the amplification of most genes, if the amplified fragments are high GC content fragments or complex structural samples, the pre-denaturation time can be increased to 2 min.
- 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.
- 3) Melting curve: Usually the instrument default program is used.

### 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.