

## DESCRIPTION

This product is a Taq DNA polymerase-based 2× master mix for realtime PCR, which contains all components, except for the primer. This reagent is applicable for intercalation assay with SYBR Green I. This reagent can be used in glass capillary systems or passive reference system. Hot Start technology with anti-Taq DNA polymerase antibodies enables high specificity and reproducible amplification.

## COMPONENTS

QST-100 can be used for 1000 reactions for a total 20μL reaction volume.

Cat NO.	Components	Size
QST-100	2×TOROGreen <sup>®</sup> qPCR Master Mix	1 mL × 10tubes/ Kit

## PRIMER DESIGN

- **Primer length:** 20~30bp
- **GC content of primer:** 40~80%
- **Target length:** ≤ 200 bp (optimally, ≤ 150bp)

## SPECIMEN

-**cDNA** : Reverse transcription reactions from total or poly (A)<sup>+</sup> RNA may be used directly, or after dilution, for realtime PCR. Purified RNA by phenol/ chloroform extraction and ethanol precipitation may also be used. Oligo dT and random primers are suitable for the reverse transcription reaction.

-**Genomic DNA:** Purified DNA, which would be used for general PCR, is also suitable for real-time PCR. In the case of mammalian genomic DNA, 1~10 ng genomic DNA is sufficient for real-time PCR.

## PROTOCOL

-This premix should be fully thawed at room temperature in the bags, gently vortexed and briefly centrifuged.

**Notes:** Due to the high concentration stabilizer, there may be crystal precipitation in the premix, which can be used normally after being fully thawed at room temperature. **If used within 6 months, it should be stored at 2-8°C in the dark bag.**

-Purified DNA and RT reactions can be may be used directly or after dilution.

-In order to reducing the artificial error of sampling, design the plate layout and sampling method by the number of the templates and primer pairs. According to the following two situations, the total reaction is divided into two parts for premixing and loading in the a thin-walled qPCR tube or plate at room temperature.

### Fore more genes and less samples in one plate

Components	20μL reaction ×n	Operation
TOROGreen <sup>®</sup> qPCR Master Mix	10μL ×n	Premix and Loading
Template DNA Dilutions	2μL ×n	
2μM Forward primer	4μL ×n	Premix and Loading
2μM Reverse primer	4μL ×n	

### Fore more samples and less genes in one plate

Components	20μL reaction ×n	Operation
TOROGreen <sup>®</sup> qPCR Master Mix	10μL ×n	Premix and Loading
8μM Reverse primer	1μL ×n	
8μM Reverse primer	1μL ×n	
Template DNA Dilutions	8μL ×n	Premix and Loading

-Gently mix the reaction solutions and spin down in microcentrifuge.

### CYCLING CONDITIONS

2-step PCR protocol				
1	Pre-denaturation	95°C	60 sec	1 cycle
2	Denaturation	95°C	10 sec	40 cycles
	Annealing/ Extension	60°C	30 sec	
Data collection should be performed at the extension step.				

3-step PCR protocol				
1	Pre-denaturation	95°C	60 sec	1 cycle
2	Denaturation	95°C	15 sec	40 cycles
	Annealing	55-65°C	15 sec	
	Extension	72°C	45 sec	
Data collection should be performed at the extension step.				

#### Notes:

-Use this protocol first and optimize PCR conditions when necessary. Perform 3-step PCR when using primers with low  $T_m$  values or when 2-step PCR is not feasible.

-The annealing temperature can be set to 55~65°C, depending on the primer  $T_m$  value.

-The annealing time should be set for 5~20 seconds. Longer annealing time results increased efficiency, and a shorter time decreases non-specific amplification.

-The pre-denaturation condition described above is sufficient for inactivation of the anti-Taq DNA polymerase antibodies used in Hot Start PCR. To prevent unexpected and inappropriate results, do not prolong the pre-denaturation period.

-Data collection step should be longer than 10 sec.

-If commercially available primers are employed, the recommended conditions from each company should be used.

### DETECTION

-This reagent can be used in general detection devices, not needing ROX such as: LineGene(bioer); LightCycler (Roche); iCycler iQ, CFX96 (Biorad/MJ); Thermal Cycler Dice (Takara);

-This reagent with 1× ROX can also be used in detection equipment using passive reference, such as: ABI PRISM 7000, 7700, 7900, 7300; Step One, Step one plus etc. (ABI), ABI PRISM 7500, 7500Fast (ABI); Mx3000P, 3005P, MX4000, etc. (Agilent).

### STORAGE

This reagent can be stored at 2-8°C for 6 months and protected from light. For longer storage, this reagent should be kept at -20°C and protected from light.