2. qPCR reaction procedure
   Please thaw and mix the reagents before configuration.

| Steps | Temperature | Time | Cycle Number |
|---|---|---|---|
| Pre-denaturation [1)] | 95℃ | 30 s | 1 |
| Denaturation [2)] | 95℃ | 5-15 s | 40-45 |
| Annealing/Extension [3)] | 60℃ | 10-30 s * | |
| | 95℃ | 15 s | |
| Melting Curve [4)] | 60℃ | 1 min * | |
| | 95℃ | 1s | |

**Note: 1) This pre-denaturing condition is suitable for most amplification reactions. The standard procedure can choose 30 s, and the shortest option for fast procedure can be 5 s. If the template structure is complex, the pre-denaturation time can be extended to 3 min to improve the pre-denaturation effect.**

**2) The standard procedure can choose 10 s, and the shortest option for rapid procedure can be 5 s.**

**3) The standard procedure can choose 30 s. For fast procedure, the shortest option can be 10 sec for amplicons within 200 bp, and 30 sec is recommended for amplicons over 200 bp.**

**4) Please set the recommended program of the fluorescence PCR instrument used for the analysis of the melting curve. This procedure is set with the ABI-Q5 fluorescence quantitative PCR instrument as the reference.**

**Set the signal acquisition at the * mark.**





# SuperStar Universal SYBR Master Mix

**Catalog Number:** CW3360

## Product Introduction

SuperStar Universal SYBR Master Mix is a dedicated master mix for dye-based (SYBR Green I) real-time qPCR reactions with a buffer optimized for qPCR at a concentration of 2×. The ROX Reference Dye included in this product is suitable for most qPCR instruments. This product is not prone to bubble formation when shaken, and only need to add primers and templates during the reaction, and the operation is simple and convenient. The core ingredient, SuperFastStar DNA Polymerase, is an antibody-based hot-start DNA polymerase that restores DNA polymerase activity after heating at 95℃ for 5 seconds. It has the advantages of strong specificity and high sensitivity, and is compatible with rapid amplification programs and small system amplification.

**Storage Condition:** -20℃, away from light.

## Components

| Component | CW3360S | CW3360M | CW3360H |
|---|---|---|---|
| 2×SuperStar Universal SYBR Master Mix | 1 mL | 5×1 mL | 40×1 mL |
| dd$H_2O$ | 1 mL | 5×1 mL | 40×1 mL |

## Precautions

1. Mix gently upside down before use, and centrifuge it instantaneously before use.
2. This product should avoid repeated freeze-thaw. The frequency of freeze-thaw should be less than 10 times, so as not to cause a decrease in enzyme activity.
3. Due to the high detection sensitivity of this product, it may be easy to be contaminated by aerosols in the air. Therefore, the preparation of the reaction system should be carried out in the ultra-clean bench. Sterilized pipette and reaction tube should be used during the preparation process. Special pipette and pipette head with filter are recommended for laboratories where conditions permit.
4. Because this product contains fluorescent dye SYBR Green I, strong light exposure should be avoided as much as possible when preparing the reaction system.

## Template Preparation

cDNA:

We recommend using our HiFiScript All-in-one RT MasterMix for qPCR (CWBIO#CW3371) to reverse transcribe RNA into cDNA. For qPCR detection, primers or reverse transcriptase during cDNA synthesis will have an impact on PCR, so it is recommended to dilute the template more than 10 times. If the stock solution is used for detection, it is recommended that the amount of template addition does not exceed 10% of the total reaction system.

gDNA:

Please use the purified sample for ordinary PCR. If it is human genome DNA, 1 to 10 ng is appropriate. If a large amount of genomic DNA is added, the signal of the genome itself will be detected, causing the baseline to drift.



## Protocol

The following examples show the conventional qPCR reaction system and reaction conditions, which should be improved and optimized according to the different templates, primer structures, and target fragment sizes.

1. qPCR reaction system

| Reagent | 20 μL System | Final Concentration |
|---|---|---|
| 2×SuperStar Universal SYBR Master Mix [1)] | 10 μL | 1× |
| Forward Primer，10 μM [2)] | 0.4 μL | 0.2 μM |
| Reverse Primer，10 μM [2)] | 0.4 μL | 0.2 μM |
| Template DNA [3)] | X μL | |
| dd$H_2O$ | to 20 μL | |

**Note: 1) 2×SuperStar Universal SYBR Master Mix is set as 1/2 of the reaction system, and other components are adjusted according to the optimal conditions. If the total volume of the reaction system needs to be changed, the other components should also be changed in the same proportion.**

**2) Generally, a better result can be obtained with a primer concentration of 0.2 μM. Final concentration of 0.1-1.0 μM can be set as a reference. The primer concentration can be increased when the amplification efficiency is not high, and the primer concentration can be reduced in the case of non-specific reactions.**

**3) Generally, the amount of DNA template is 1-100 ng genomic DNA or 1-10 ng cDNA as reference. Because template contains different copies of the target gene in different species, the template can be gradient diluted to determine the optimal amount. If the template type is an undiluted cDNA stock, the volume used should not exceed 1/10 of the total volume of the qPCR reaction.**

**4) In the actual preparation, the other components except the sample solution can be premixed into a mixture of n+α times (n is the number of samples, α is the packaging loss), and then divided into each tube (n tubes in total), and finally add the corresponding sample solution in each tube.**