

## 2× GoldStar Best MasterMix

### Catalog Number:

CW0656S (1 mL)  
CW0656M (5 mL)  
CW0656L (25 mL)

### Storage Condition:

-20 °C; For frequent uses, store at 2-8 °C.

### Kit Components:

Component	CW0656S (1 mL)	CW0656M (5 mL)	CW0656L (25 mL)
2× GoldStar Best MasterMix	1 mL	5× 1 mL	5× 5 mL
ddH <sub>2</sub> O	1 mL	5× 1 mL	5× 5 mL

**Note: 2× GoldStar Best MasterMix contains GoldStar Best DNA Polymerase, 3.4 mM MgCl<sub>2</sub> and 400 μM each dNTP.**

## Product Introduction:

This product is a premixed system composed of GoldStar DNA Polymerase, Mg<sup>2+</sup>, dNTPs, and PCR stabilizers and enhancers at a concentration of 2x. This product has the advantages of simple and quick operation, high sensitivity, high specificity and good stability, which decrease human error and contamination maximumly. The GoldStar Best DNA polymerase has 5'-3' DNA polymerase activity, 5'-3' exonuclease activity and 3'-5' exonuclease activity. Compared with GoldStar DNA Polymerase, this polymerase has higher amplification efficiency and lower mismatch rate under normal PCR conditions. The chemical-modified enzyme does not have polymerase activity at room temperature, thus effectively avoiding non-specific amplification. The polymerase must be activated by incubation at 95 °C for 10 minutes.

The optimized buffer system maximizes the effect of the enzyme and achieves high fidelity, high specificity, high amplification efficiency, and high sensitivity amplification of the target fragment. This product does not contain dyes, and an appropriate amount of sample loading buffer should be added for electrophoresis. Most of the amplified PCR products have an "A" base attached to the 3' end, and therefore can be directly used for T/A cloning. It is mainly suitable conventional PCR reactions and experiments such as gene cloning that require high fidelity.

## Quality Control:

No exogenous nuclease activity was detected. No host DNA was detected by PCR. Single copy genes in multiple genomes could be efficiently amplified. No apparent activity change after being stored at 2-8 °C for three months.

## Protocol:

The following is an example of a PCR reaction system and reaction conditions for amplifying a 1 kb fragment using human genomic DNA as a template. The actual operation should be based on the template, the structure of the primers, and the size of the target fragment to make corresponding improvements and optimizations.

### 1. PCR reaction system:

Reagent	50 µL	Final Conc.
2× GoldStar Best MasterMix	25 µL	1×
Forward Primer, 10 µM	2 µL	0.4 µM
Reverse Primer, 10 µM	2 µL	0.4 µM
DNA template	< 0.5 µg	< 0.5 µg/50 µL
ddH <sub>2</sub> O	Up to 50 µL	

**Note:** For the primer concentration, please refer to the final concentration of 0.1-1.0 µM as a reference for the setting range. When the amplification efficiency is not high, the concentration of the primer can be increased; when a non-specific reaction occurs, the concentration of the primer can be reduced, thereby optimizing the reaction system.

### 2. PCR reaction program:

Step	Temperature	Time	Cycles
Initialization	95 °C	10 min	1
Denaturation	94 °C	30 sec	
Annealing	55-65 °C	30 sec	30-40
Extension	72 °C	1 min	
Final Extension	72 °C	5 min	1

## Note:

- 1) In general, the annealing temperature is 5 °C lower than the melting temperature (T<sub>m</sub>) of the primer. When the ideal amplification efficiency cannot be obtained, the annealing temperature should be appropriately lowered; when non-specific reactions occur, the annealing temperature should be increased, thereby optimizing the reaction conditions.
- 2) The extension time should be set according to the size of the amplified fragment. The amplification efficiency of the GoldStar Best DNA Polymerase is 1-2 kb/min.
- 3) The number of cycles can be set according to the downstream application of the amplification product. If the number of cycles is too small, the amount of amplification is insufficient. If the number of cycles is too big, the probability of mismatch increases, and the non-specific background is severe. Therefore, the number of cycles should be reduced as much as possible yet ensuring the yield of the product.
- 4) This product must be activated by incubation at 95 °C for 10 minutes.