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# 2×GoldStar MasterMix (Dye)

**Cat. No. :** CW0960S (1 mL)
CW0960M (5 mL)

**Storage Condition:** -20 ℃. For frequent use, it can be stored at 2-8 ℃.

## Components

| Component | CW0960S<br>1 mL | CW0960M<br>5 mL |
|---|---|---|
| 2×GoldStar MasterMix (Dye) | 1 mL | 5×1 mL |
| $ddH_2O$ | 1 mL | 5×1 mL |

**Note: 2×GoldStar MasterMix contains GoldStar DNA Polymerase, 3.4 mM $MgCl_2$ and 400 μM each dNTP.**

## Principle

2×GoldStar MasterMix is a premix system composed of GoldStar DNA Polymerase, $Mg^{2+}$, dNTPs, PCR stabilizer and reinforcer. It has the advantages of simple and rapid operation, and can minimize human error and contamination. The GoldStar DNA Polymerase included in the product is a chemically modified Taq DNA polymerase. The chemically modified enzyme has no polymerase activity at room temperature and is effective in avoiding the nonspecific amplification caused by the nonspecific binding of primer and template or primer dimer at room temperature. The activation of the enzyme requires incubation at 95 ℃ for 10 min. The unique buffer system makes the enzyme suitable for more application, so that the template with high GC content, complex secondary structure and low copy can be efficiently amplified, and the unique MasterMix formula makes the whole reaction system more stable. This product has been added dye (blue), and can be directly electrophoresis detected after the reaction. Most PCR products obtained by amplification have an "A" base attached to the 3 'end, so they can be directly used for T/A cloning. This product has strong specificity, and does not require purification to remove impurity after PCR amplification, and can be directly used in downstream cloning or chip hybridization experiments. It is suitable for conventional PCR, RT-PCR and multiplex PCR experiments, and is especially suitable for PCR reactions with high specificity requirements.

## Quality Control

No exogenous nuclease activity was detected. The PCR method detected no residual host DNA. It can efficiently amplify single-copy genes from various genomes. There was no obvious change in activity when stored at 2-8 ℃ for 3 months.

## Procedure

The following examples show the reaction system and reaction conditions for the amplification of 1 kb fragment using human genomic DNA as template, which should be improved and optimized according to the different templates, primer structures, and target fragment sizes.

1. PCR Reaction System

| Reagent | 50 μL Reaction system | Final Concentration |
|---|---|---|
| 2×GoldStar MasterMix (Dye) | 25 μL | 1× |
| Forward Primer，10 μM | 2 μL | 0.4 μM |
| Reverse Primer，10 μM | 2 μL | 0.4 μM |
| Template DNA | <0.5 μg | <0.5 μg/50 μL |
| $ddH_2O$ | to 50 μL | |

**Note: The final concentration of 0.1-1.0 μM can be set as a reference for setting range. 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, so that the reaction system can be optimized.**

2. PCR reaction condition

| Step | Temperature | Time | |
|---|---|---|---|
| Pre-denaturation | 95 ℃ | 10 min | |
| Denaturation | 95 ℃ | 30 s | 30-40 cycles |
| Annealing | 55-65 ℃ | 30 s | |
| Extension | 72 ℃ | 60 s | |
| Final extension | 72 ℃ | 5 min | |

**Note:**

**1) In general experiments, the annealing temperature is 5 ℃ lower than the melting temperature (Tm) of the amplification primer, and the annealing temperature should be appropriately reduced when the ideal amplification efficiency cannot be obtained. When nonspecific reactions occur, increase the annealing temperature to optimize reaction conditions.**

**2) The extension time should be set according to the size of the amplified fragment. The amplification efficiency of the GoldStar Taq DNA Polymerase included in this product 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 low, there is insufficient amplification. If the number of cycles is too high, the mismatch rate increases, leading to significant nonspecific background. Therefore, the number of cycles should be minimized on the premise of ensuring the yield of the product**

**4) The product must be pre-denaturated at 95 ℃ for 10 min to activate the enzyme.**