# 2x GoldStar Best PCR Master Mix (with dye)

## Cat. #: W0655-1 (1 mL); W0655-5 (5 mL)

Storage: Ship at 4°C. Store at -20°C for up to 1 year and avoid freeze-thaw cycles. Store at 4°C for up to 3 months.

#### **Product Components**

Components	W0655-1	W0655-5
2x GoldStar Best PCR Master Mix	1 mL	1 mL x 5
RNase-Free Water	1 mL	5 mL

**Note:** 2x GoldStar Best PCR Master Mix contains GoldStar Best DNA Polymerase, 2x GoldStar Best PCR Buffer, 3 mM MgCl2 and 400 μM dNTPs.

## Application

- Routine PCR
- Ideal for high fidelity gene cloning

## Product Description (This product is for research use only.)

2x GoldStar Best PCR Master Mix is a **convenient** premixed 2x concentrated solution for PCR which include GoldStar Best DNA Polymerase, PCR Buffer, Mg2+, dNTPs, PCR Stabilizer and PCR Intensifier.

The polymerase has a  $5' \rightarrow 3'$  DNA polymerase and a  $5' \rightarrow 3'$  exonuclease activity. It also possesses  $3' \rightarrow 5'$  exonuclease (proofreading) activity. The polymerase has a **higher amplification efficiency and lower mispairing rate** than routine Taq DNA polymerase.

The GoldStar Best is a high **reliable hotstart** polymerase, which activity is inhibited at ambient temperatures by the chemical modification. This prevents the formation of misprimed products and primer dimers at ambient temperatures. GoldStar Best Polymerase is activated by a 10 minutes, 95°C incubation. Optimized buffer system promotes the amplification of target fragment with **high fidelity**, **high specificity**, **high amplification efficiency and high sensitivity**. GoldStar Best DNA Polymerase catalyzes the non-template directed addition of an adenine residue to the 3'-end of both strands of DNA molecules to make it suitable for **TA cloning**. The amplification range of Es Taq is ~ 6 kb. The Master Mix contains dyes, and can directly run electrophoresis after PCR reaction.

#### **Quality control:**

This product is tested for no exogenous nuclease activity; no host DNA contamination tested (by PCR); able to amplify single copy gene from multiple genomes; and no significant enzyme activity decrease after storing at 2 ~ 8°C for 3 months.

#### Protocol

The following protocol serves as a general guideline for routine PCR amplification. Optimal reaction conditions (annealing temperature and time, extension time, concentration of primers and template DNA) vary and need to be optimized according to the template, primer structure and target fragment size.

1. Prepare the reaction mix to 50  $\mu$ L according to the following table.

Reagent	50 µl PCR reaction	Final Concentration
2x GoldStar Best PCR Master Mix	25 µl	1x
Forward Primer, 10 µM	2 µl	0.4 µM
Reverse Primer, 10 µM	2 µl	0.4 µM
Template DNA	<1 µg	<1 µg / reaction
RNase-Free Water	Up to 50 µl	-

**Note:** The recommended primer concentration for PCR is between 0.1-1.0 µM of each primer. The use of higher concentrations of primers can have higher amplification effect. Low primer concentration will generally ensure cleaner product and lower background.

#### 2. PCR reaction conditions

Procedure	Temperature	Time	
Pre-denaturation	<b>95</b> ℃	10 min	
Denaturation	<b>94</b> °C	30 s	
Annealing	<b>55-65</b> ℃	30 s 👌 🤅	30-40 cycles
Extension	<b>72</b> °C	60 s	
Final extension	<b>72</b> °C	2 min	

#### Note:

- The recommended annealing temperature is about 5°C below Tm of primers. If non-specific bands are observed, increase the annealing temperatures. The absence of product indicates the need for a lower annealing temperature.
- PCR extension time is depended on the size of target gene sequence. The efficiency of GoldStar Best Taq DNA polymerase is approximately 1 kb DNA / 60 seconds.
- 3) The number of PCR cycles will basically depend on the downstream application of the PCR product.
- 4) GoldStar Best Polymerase requires to be activated by a 10 minutes, 95°C incubation
- 3. PCR result examination

This Master Mix contains dyes for electrophoresis. After PCR, directly load 5 µL of PCR product to agarose gel to run electrophoresis. No need to add loading buffer.

-- The end --