

2×GoldStar Best MasterMix (Dye)

Item No. G665556 (5 ml)

Storage conditions: -20° C. If frequent use is required, store at 2-8° C.

Product content

individual parts making up a compound	G665556 5 ml
2×GoldStar Best MasterMix (Dye)	5 x 1 ml
ddH ₂ O	5 x 1 ml

Product Introduction

This product is a premixed system consisting of GoldStar Best DNA Polymerase, Mg²⁺, dNTPs, and PCR stabilizers and enhancers at a concentration of 2×. It has the advantages of easy and fast operation, high sensitivity, high specificity, and good stability, which can minimize human error and contamination. The GoldStar Best DNA Polymerase contained in this product is a chemically modified hot-start high-fidelity polymerase. The polymerase has 5' → 3' DNA polymerase activity, 5' → 3' exonuclease activity and 3' → 5' exonuclease activity, and has the excellent performance of high amplification efficiency and low mismatch rate compared with GoldStar Taq DNA Polymerase under normal PCR conditions. The chemically modified enzyme has no polymerase activity at room temperature, which effectively avoids non-specific amplification caused by non-specific binding of primers and templates or primer dimerization at room temperature, and the enzyme activation must be incubated at 95° C for 10 min, which can be integrated into existing PCR thermal cycling programs. The optimized buffer system allows the enzyme to function with maximum efficacy, achieving high fidelity, high specificity, high amplification efficiency and high sensitivity amplification of the target fragment. The product has incorporated dye (blue color), which can be directly detected by electrophoresis after the reaction. Most of the amplified PCR products have an "A" base at the 3' end, so they can be directly used for T/A cloning. It is suitable for routine PCR reaction and gene cloning which requires high fidelity.

Quality control

No exogenous nuclease activity was detected; no host residual DNA was detected by PCR; single-copy genes in various genomes could be amplified efficiently; no significant change in activity after storage at 2-8° C for three months.

Usage

The following is an example of a PCR reaction system and reaction conditions for amplifying a 1 kb fragment of human genomic DNA as a template, which should be improved and optimized according to the template, primer structure and size of the target fragment in actual operation.

1. PCR reaction system

reagents	50 μ l reaction system	final concentration
2 \times GoldStar Best MasterMix (Dye)	25 μ l	1 \times
Forward Primer, 10 μ M	2 μ l	0.4 μ M
Reverse Primer, 10 μ M	2 μ l	0.4 μ M
Template DNA	<0.5 μ g	<0.5 μ g/50 μ l
ddH ₂ O	up to 50 μ l	/

Note: Please use the final concentration of 0.1-1.0 μ M as a reference for setting the range of primer concentration. If the amplification efficiency is not high, the primer concentration can be increased; if a non-specific reaction occurs, the primer concentration can be decreased to optimize the reaction system.

2. PCR reaction conditions

move	temp	timing	/
premutability	95° C	10 min	/
denaturation	94° C	30 s	30-40 cycles
annealing (metallurgy)	55-65° C	30 s	30-40 cycles
reach	72° C	60 s	30-40 cycles
At last, an extension.	72° C	5 min	/

Attention:

(1) In general, the annealing temperature is 5°C lower than the melting temperature of the amplification primer T_m . When the ideal amplification efficiency cannot be obtained, the annealing temperature should be lowered appropriately; when a non-specific reaction occurs, the annealing temperature should be increased, thus optimizing the reaction conditions.

(2) The extension time should be set according to the size of the amplified fragments. The amplification efficiency of GoldStar Best 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 amplified product. If the number of cycles is too low, the amount of amplification will be insufficient; if the number of cycles is too high, the chance of mismatch will increase and the non-specific background will be serious. Therefore, the number of cycles should be minimized under the premise of ensuring the product yield.

(4) The enzyme should be activated under the condition of pre-denaturation at 95°C for 10 min.