Tinzyme Co., Limited

Email: sales@tinzyme.com Website: www.tinzyme.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

2×HotStar Best MasterMix (Dye)

Product Number: PCM56B

Shipping and Storage

-20℃. For frequent use, it can be stored at 2-8℃

Components

Component	PCM56B 5mL
2×HotStar Best MasterMix (Dye)	5×1mL
ddH_2O	5×1mL

Note: The 2×HotStar Best MasterMix (Dye) contains HotStar Best DNA Polymerase,3.4mM $MgCl_2$ and 400μM each dNTP.

Description

This product is a premixed system composed of HotStar Best DNA Polymerase, Mg^{2+}, dNTPs, PCR stabilizers and enhancers, with a concentration of 2×,It has the advantages of simple and fast operation, high sensitivity, strong specificity, and good stability, which can minimize human error and pollution to the greatest extent. The HotStar Best DNA Polymerase contained in this product is a chemically modified hot start high fidelity polymerase.This polymerase has 5'-3' DNA polymerase activity, 5'-3' exonuclease activity, and 3'-5' exonuclease activity. Under ordinary PCR conditions, compared with GoldenStar Taq DNA polymerase, it has excellent performance of high amplification efficiency and low mismatch rate.The chemically modified enzyme has no polymerase activity at room temperature, effectively avoiding non-specific amplification caused by non-specific binding of primers and templates or primer dimers at room temperature. The activation of the enzyme must be incubated at 95℃ for 10 minutes, which can be integrated into existing PCR thermal cycling programs.The optimized buffer system maximizes the effectiveness of the enzyme, achieving high fidelity, specificity, amplification efficiency, and sensitivity for the target fragment.This product has been added with a dye (blue) and can be directly subjected to electrophoresis detection after the reaction is completed.Most of the PCR products obtained by amplification have an "A" base attached to the 3'end, so they can be directly used for T/A cloning. Suitable for routine PCR reactions and gene cloning experiments with high fidelity requirements.

Quality Control

After testing, there was no exogenous nuclease activity; PCR method for detecting non host residual DNA; Can effectively amplify single copy genes from multiple genomes; Storage at 2-8 ℃ for three months showed no significant change in activity.

Protocol

The following example is a PCR reaction system and reaction conditions for amplifying a 1kb fragment using human genomic DNA as a template. In actual operation, corresponding improvement and optimization should be carried out according to the different template, primer structure and target fragment size.

1. PCR reaction system

Reagent	50μL reaction system	Final Concentration
2×HotStar Best MasterMix (Dye)	25μL	1×
Forward Primer,10 μM	2μL	0.4μM
Reverse Primer,10 μM	2μL	0.4μM
Template DNA	< 0.5μg	< 0.5μg/50μL
ddH_2O	up to 50μL	

Note:Please use the final concentration of 0.1-1.0μM as the reference for the setting range of primer concentration. When the amplification efficiency is not high, the concentration of primers can be increased; when non-specific reactions occur, the

concentration of primers can be decreased to optimize the reaction system.

2. PCR reaction condition

Step	Temperature	Time	
predenaturation	95°C	3min	
denaturation	94°C	30s	30-40cycles
annealing	55-65°C	30s	
extend	72°C	60s	
final extension	72°C	5min	

Note:1) In general experiments, the annealing temperature is 5°C lower than the melting temperature Tm of the amplification primer. When the ideal amplification efficiency cannot be obtained, the annealing temperature should be appropriately reduced; when non-specific reactions occur, the annealing temperature should be increased to optimize the reaction Conditions.

2) The extension time should be set according to the size of the amplified fragment. The amplification efficiency of HotStar Best DNA Polymerase contained 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 small, the amount of amplification will be insufficient; if the number of cycles is too many, the probability of mismatching will increase and the non-specific background will be severe. Therefore, the number of cycles should be minimized on the premise of ensuring the product yield.

4) This product must be pre-denatured at 95°C for 10 min to activate the enzyme.