Tinzyme Co., Limited

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2×Hotstar PCR MasterMix

Product Number: PCM13

Shipping and Storage

-20°C. Store at 2-8°C for frequent use.

Components

Component	PCM13 5mL	PCM13 25mL
2×Hotstar PCR MasterMix	5×1mL	5×5mL
ddH_2O	5×1mL	5×5mL

Description

2×Hotstar PCR MasterMix is a premixed system consisting of Golden Star Taq DNA Polymerase, PCR Buffer, Mg^{2+}, dNTPs, PCR stabilizers and reinforcers. The premixed PCR mixture makes the operation easier and faster. Minimizes human error and pollution. The Golden Star Taq DNA Polymerase is a chemically modified, new highefficiency Taq DNA Polymerase that completely blocks the enzyme activity at room temperature, making the enzyme inactive at low or normal temperatures. In order to effectively avoid the non-specific amplification caused by the non-specific combination of primer and template or primer dimer at room temperature, the activation of the enzyme must be incubated at 95°C for 10 min. The unique buffer system enables the enzyme to be widely used, enabling efficient amplification of templates with high GC content, complex secondary structure and low copy. The unique MasterMix formula makes the whole reaction system more stable. PCR amplification with this product, PCR product 3' end with an "A" base, can be directly used for T/A cloning. This product does not contain dye, PCR procedure after bunching can be added according to the need of sample loading buffer after electrophoresis operation. This product has strong specificity and can be directly used for downstream cloning or chip hybridization experiments without the need of agarose gel recovery after PCR amplification. It is mainly used for conventional PCR, RT-PCR, multiple PCR and gene chip detection, especially for PCR reaction with high specificity requirements.

Quality Control

No exogenous nuclease activity was detected. No host residual DNA was detected by PCR. It can effectively amplify single copy genes in human genome. 2-8°Cstore three months , no significant activity change.

Protocol

The following examples are the PCR reaction system and reaction conditions for the amplification of 1 KB fragment using human genomic DNA as template. In actual operation, corresponding improvements and optimization should be made according to the structure of template primers and the size of target fragment.

1. PCR Reaction System

Reagent	50μL Reaction System	Final Concentration
2×Hotstar PCR MasterMix	25μL	1×
Primer,10μM	2μL	0.4μM
Primer,10μM	2μL	0.4μM
DNA <0.5μg	<0.5μg	<0.5μg/50μL
ddH_2O	Up to 50μL	

Note:Primer concentration should take final concentration 0.1-1.0μM as reference for setting range. When the amplification efficiency is not high, the primer concentration can be increased. When nonspecific reactions occur, the concentration of primers can be reduced to optimize the reaction system.

2. PCR Reaction Condition

Step	Temperature	Time	
Predenaturation	95°C	10min	
Denaturation	95°C	30s	30-40cycles
Annealing	55-65°C	30s	
Extend	72°C	60s	
Final extend	72°C	5min	

Note:1) In general experiments, the annealing temperature is 5°C lower than the melting temperature Tm of the amplification primer, and the annealing time is generally 30-60 s. If the desired amplification efficiency cannot be obtained, the annealing temperature should be appropriately reduced; When nonspecific reaction occurs, the annealing temperature isincreased to optimize the reaction strip.

2) The extension time should be set according to the size of the amplified fragment. The amplification efficiency of the Golden Star Taq DNA Polymerase included in this product is 1-2 KB /min.

3) Cycle number can be set according to downstream application of amplified products. If the number of cycles is too small, the increment of expansion is insufficient; If the number of cycles is too high, the mismatch rate will increase, and the nonspecific background will be serious. Therefore, the number of cycles should be reduced as far as possible under the premise of ensuring the product yield.

4) The product should be pre-denaturated at 95°C for 10 min to achieve enzyme activation.