Tinzyme Co., Limited

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2×HotStar Best MasterMix

Product Number: PCM56

Shipping and Storage

-20°C; For frequent uses, store at 2-8°C.

Component

Component	PCM56 1ml	PCM56 5ml	PCM56 25ml
2×HotStar Best MasterMix	1mL	5×1mL	5×5mL
ddH_2O	1mL	5×1mL	5×5mL

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

Description

This product is a premixed system composed of HotStar Best MasterMix, Mg^{2+}, dNTPs, and PCR stabilizers and enhancers at a concentration of 2×. This product has the advantages of simple and quick operation, high sensitivity, high specificity and good stability, which decrease human error and contamination maximumly. The HotStar Best DNA Polymerase has 5'-3' DNA polymerase activity, 5'-3' exonuclease activity and 3'-5' exonuclease activity. Compared with HotStar Best MasterMix, this polymerase has higher amplification efficiency and lower mismatch rate under normal PCR conditions. The chemical-modified enzyme does not have polymerase activity at room temperature, thus effectively avoiding non-specific amplification. The polymerase must be activated by incubation at 95°C for 10 minutes.

The optimized buffer system maximizes the effect of the enzyme and achieves high fidelity, high specificity, high amplification efficiency, and high sensitivity amplification of the target fragment. This product does not contain dyes, and an appropriate amount of sample loading buffer should be added for electrophoresis. Most of the amplified PCR products have an "A" base attached to the 3' end, and therefore can be directly used for T/A cloning. It is mainly suitable conventional PCR reactions and experiments such as gene cloning that require high fidelity.

Quality Control

No exogenous nuclease activity was detected; No host DNA was detected by PCR; single copy genes in multiple genomes could be efficiently amplified; No apparent activity change after being stored at 2-8°C for three months.

Protocol

The following is an example of a PCR reaction system and reaction conditions for amplifying a 1kb fragment using human genomic DNA as a template. The actual operation should be based on the template, the structure of the primers, and the size of the target fragment to make corresponding improvements and optimizations.

1. PCR reaction system

Reagent	50μL	Final Conc.
2×HotStar Best MasterMix	25μL	1×
Forward Primer,10μM	2μL	0.4μM
Reverse Primer,10μM	2μL	0.4μM
DNA template	< 0.5μg	< 0.5μg/50μL
ddH_2O	up to 50μL	

Note: For the primer concentration, please refer to the final concentration of 0.1-1.0μM as a reference for the setting range. When the amplification efficiency is not high, the concentration of the primer can be increased; when a non-specific reaction occurs, the concentration of the primer can be reduced, thereby optimizing the reaction system.

2. PCR reaction condition

Step	Temperature	Time	Cycles
Pre denaturation	95℃	10min	
Denaturation	94℃	30s	
Annealing	55-65℃	30s	30-40cycles
Extend	72℃	60s	
Final extension	72℃	5min	

Note:1) In general, the annealing temperature is 5°C lower than the melting temperature (Tm) of the primer. When the desired amplification efficiency cannot be obtained, the annealing temperature is appropriately lowered; when non-specific reactions occur, the annealing temperature is increased, thereby optimizing the reaction conditions.

2) The extension time should be set according to the size of the amplified fragment. The amplification efficiency of the HotStar Best DNA Polymerase is 1-2kb/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 small, the amount of amplification is insufficient; if the number of cycles is too big, the probability of mismatch increases, and the non-specific background is severe. Therefore, the number of cycles should be reduced as much as possible yet ensuring the yield of the product.

4) This product must be activated by incubation at 95°C for 10 minutes.