Tinzyme Co., Limited

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2×E Taq Master mix, for PAGE

Product Number: PCM12A

Shipping and Storage

-20℃。

Components

Component	PCM12A 1ml	PCM12A 5ml	PCM12A 25ml
2×E Taq Master mix, for PAGE	1ml	5×1ml	5×5ml
ddH_2O	1ml	5×1ml	5×5ml

Note: 2×E Taq Master mix, for PAGE contains E Taq DNA Polymerase, 3mM $MgCl_2$ and 400μM each dNTP

Description

This product is a premixed system consisting of E Taq DNA Polymerase, Mg^{2+}, dNTPs, PCR stabilizer and enhancer at a concentration of 2×. E Taq DNA Polymerase has excellent properties of high amplification efficiency and low mismatch rate. The original MasterMix formula makes the whole reaction system very stable, the success rate of PCR amplification reaction is more than 98% of PCR amplification can be successful in one time, while complex templates can be amplified effectively, and can minimize human error and contamination. This product does not contain dye. After PCR procedure, appropriate amount of loading buffer can be added as required for electrophoresis operation. PCR products can be used directly for T/A cloning because most PCR products obtained by amplification are attached to the "A" base at the 3' end. It is mainly suitable for routine PCR reaction and gene cloning experiments which require high fidelity. The PCR amplified products are specially used for polyacrylamide coagulation detection.

Quality Control

No exogenous nuclease activity was tested. No host residual DNA was detected by PCR. It can effectively amplify single copy genes in multiple genomes.

Protocol

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

1. PCR Reaction System

Reagent	50μL	Final Conc.
2×E Taq Master mix, for PAGE	25μL	1×
Forward Primer, 10μM	2μL	0.4μM
Reverse Primer, 10μM	2μL	0.4μM
Template DNA	<0.5μL	<0.5μg/50μl
ddH_2O	up to 50μL	

Note: Primer concentration please use final concentration 0.1-1.0μM as reference for the set range. The concentration of primers can be improved if the amplification efficiency is not high. When non-specific reaction occurs, the concentration of primers can be reduced to optimize the reaction system.

2. PCR Reaction Condition

Step	Temperature	Time
Initial denaturation	94℃	2min

Denaturation	94℃	30s	
Annealing	55-65℃	30s	25-35cycles
Extension	72℃	30s	
Final Extension	72℃	2min	

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

2) The elongation time should be set according to the amplified fragment size. The amplification efficiency of E Taq DNA Polymerase is 2kb/min.

3) The cycle number can be set according to the downstream application of the amplified product. If the number of cycles is too small, the increment of expansion is insufficient; If the number of cycles is too large, the likelihood of mismatch will increase and the non-specific background will be severe. Therefore, the number of cycles should be reduced as far as possible under the premise of ensuring the yield of products.