Tinzyme Co., Limited

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2×E Taq Master mix, with blue dye

Product Number: PCM12B

Shipping and Storage

-20°C

Components

Component	PCM12B (1mL)	PCM12B(5mL)	PCM12B(25mL)
2×E Taq Master mix, with blue dye	1mL	5×1mL	5×5mL
ddH_2O	1mL	5×1mL	5×5mL

Note:2×E Taq Master mix, with blue dye contains E Taq DNA Polymerase , 3mM $MgCl_2$ and 400μM each dNTP.

Description

This product is a premixed system composed of E Taq DNA Polymerase, Mg^{2+}, dNTPs, and PCR stabilizers and enhancers at a concentration of 2x. E Taq DNA Polymerase has high amplification efficiency and low mismatch rate. The original MasterMix formula makes the whole reaction system very stable. More than 98% of PCR amplifications can be successful at one time. At the same time, complicated templates can be effectively amplified, and human error and contamination can be minimized. This product has been added with dye (blue), and electrophoresis gel detection can be performed directly after the reaction. 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.

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 reaction	Final Conc.
2×E Taq Master mix, with blue dye	25μL	1×
Forward primer (10μM)	2μL	0.4 μM
Reverse primer (10μM)	2μL	0.4 μM
Template DNA	<0.5μg	<0.5 ug/50 μL
ddH_2O	Up to 50 μL	

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 nonspecific reaction occurs, the concentration of the primer can be reduced, thereby optimizing the reaction system.

2. PCR reaction condition

Step	Temperature	Time	
Initialization	94°C	2mins	
Denaturation	94°C	30s	25-35cycles
Annealing	55-65°C	30s	
Elongation	72°C	30s	

Final elongation	72°C	2mins

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 E Taq DNA Polymerase is 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.