2×Pfu MasterMix (Dye)

Item No. P665594 (5 mL)
Storage conditions: -20° C. If frequent use is required, store at 2-8° C.

Product content

individual parts making up a compound	P665594 5ml
2×Pfu MasterMix (Dye)	5 x 1 ml
ddH2O	5 x 1 ml

Product Introduction

This product is a premixed system of Pfu DNA Polymerase, Mg2+, dNTPs, and PCR stabilizers and enhancers at a concentration of 2×. Pfu DNA Polymerase possesses 5′ -3′ DNA polymerase activity and 3′ -5′ exonuclease activity, and therefore has the ability to correct errors during DNA amplification. It has high fidelity (6-8 times that of Taq enzyme) and better thermal stability than Taq DNA Polymerase. Pre-formulated PCR mixes make operation easier and faster, minimizing human error and contamination. The original MasterMix formulation makes the whole reaction system very stable and reproducible. The product has added dye (blue color), which can be directly detected by electrophoresis after the reaction. The Pfu DNA polymerase contained in this product is characterized by low mismatch rate and high temperature resistance, which is suitable for gene cloning, gene spot mutation, SNP and end leveling reactions.

Quality control

No exogenous nuclease activity was detected; no host residual DNA was detected by PCR; single-copy genes in various genomes could be amplified efficiently; no significant change in activity after storage at 2-8° C for three months.

Usage

The following is an example of a PCR reaction system and reaction conditions for amplifying a 1 kb fragment of human genomic DNA as a template, which should be improved and optimized according to the template, primer structure and size of the target fragment in actual operation.

1. PCR reaction system

reagents	50 μl reaction system	final concentration
2×Pfu MasterMix (Dye)	25 μL	1×
Forward Primer, 10 μM	2 μL	0.4 μM
Reverse Primer, 10 μM	2 μl	0.4 μM
Template DNA	<0.5 μg	<0.5 μg/50 μL
ddH2O	up to 50 μL	/

Note: When amplifying with Pfu enzyme, the purity of the primer is required to be higher, the length of primer is more than 18 bases, and the concentration of primer should be 0.1-1.0 μM as the final concentration as the reference of the setting range. If the amplification efficiency is not high, the concentration of primer can be increased; if non-specific reaction occurs, the concentration of primer can be decreased to optimize the reaction system.

2. PCR reaction conditions

move	temp	timing	/
premutability	94° C	2 min	/
denaturation	94° C	30 s	25-35 cycles
annealing (metallurgy)	55-65° C	30 s	25-35 cycles
reach	72° C	60 s	25-35 cycles
At last, an extension.	72° C	5 min	/

Attention:

(1) The thermal stability of Pfu enzyme is better than that of Taq enzyme. For the template with high GC content, the denaturation temperature can be increased to 98℃, which has no effect on the activity of Pfu enzyme.
(2) In general, the annealing temperature is 5℃ lower than the melting temperature of the amplification primer Tm. When the desired amplification efficiency cannot be obtained, the annealing temperature should be lowered appropriately; when a non-specific reaction occurs, the annealing temperature should be increased, thus optimizing the reaction conditions.
(3) Pfu enzyme has 3′ -5′ exonuclease activity, so the extension speed of Pfu enzyme is much lower than Taq enzyme, the extension time is set according to the size of the amplified fragments, and the extension rate of this product is 1 kb/min.
4) The number of cycles can be set according to the downstream application of the amplified product. If the number of cycles is too low, the amount of amplification will be insufficient; if the number of cycles is too high, the chance of mismatch will increase and the non-specific background will be serious. Therefore, the number of cycles should be minimized under the premise of ensuring the product yield.
(5) This product has 3′ -5′ exonuclease activity, the PCR product is flat-ended and can not be used directly for T/A cloning, if you need to carry out T/A cloning, then you need to add "A" at the end of the product or use a flat-ended vector for cloning.