2×Flash Hot Start MasterMix (Dye)

Catalog Number:

F665646 (5 ml)

F665646 (25 ml)

F665646 (40 ml)

Storage condition: -20℃

Products content

Component	5 ml	25 ml	40 ml
2×Flash Hot Start MasterMix (Dye)	5 x 1 ml	5 x 5 ml	40 x 1 ml
ddH2O	5 x 1 ml	5 x 5 ml	40 x 1 ml

Products Introduction

This product is a premixed system consisting of a new type of highly efficient DNA polymerase, PCR Buffer, Mg2+, dNTPs, and PCR stabilizers and enhancers at a concentration of 2 ×. It contains a new type of high efficient hot starter enzyme, which can effectively inhibit the non-specific annealing of primers and non-specific amplification caused by primer dimer under low temperature. The product has a very high amplification speed and stability, the extension speed can be up to 5-15 sec/kb, suitable for rapid PCR reaction, the original MasterMix formula makes the whole reaction system very stable, more than 98% of the PCR amplification can be successful at once, at the same time, complex templates can also be amplified effectively, and can minimize the human error and contamination. The product has added dye (blue), and can be directly detected by electrophoresis after the reaction. Most of the amplified PCR products have an "A" base at the 3' end, so they can be used directly for T/A cloning. It is mainly used for rapid PCR reactions and gene cloning where high fidelity is required.

quality control

Tested to be free of exogenous nuclease activity; PCR method detects no host residual DNA; can efficiently amplify a wide range of base

Single-copy genes in the genome.

Usage

The following is an example of a PCR reaction system and reaction conditions using human genomic DNA as a template, and the actual operation should be based on the template.

Plates, primer structures and target fragment sizes were improved and optimized accordingly.

PCR reaction system

reagents	50 μl reaction system	final concentration
2×Flash Hot Start 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 ug	<0.5 μg/50 μl
ddH2O	up to 50 μl	

Note: Please use the final concentration of 0.1-1.0 μM as a reference for setting the range of primer concentration. If the amplification efficiency is not high, the primer can be increased.

concentration; when a non-specific reaction occurs, the primer concentration can be reduced, thus optimizing the reaction system.

PCR reaction conditions

move	temp	timing	
denaturation	98° C	10 sec	25-35 cycles
annealing (metallurgy)	55-65° C	5 sec	25-35 cycles
reach	72° C	5-15 sec/kb	25-35 cycles

Note: If the amplified sample is bacterial liquid, add "pre-denaturation 95℃ for 5min" step.

Optimization of parameter settings

1. Template DNA amount setting:

Excessive amounts of template may result in non-specific amplification or smear. The recommended amount of template DNA in a 50 μl PCR reaction system is as follows:

-Human genomic DNA 5 ng-500 ng

-Escherichia coli genomic DNA 50 pg-100 ng

-plasmid DNA 10 pg-1 ng

2. Primer concentration setting:

The primer concentration can be set between 0.1 μM and 1.0 μM. Too low a primer concentration may result in low amplification products. Too high a primer concentration will inhibit specific amplification and may result in non-specific amplification.

3. Annealing temperature setting: In general, the annealing temperature in the experiment is 5℃ lower than the melting temperature of the amplification primer, Tm, which is not able to get the ideal amplification efficiency.

The annealing temperature can be lowered appropriately in the case of non-specific reactions; the annealing temperature can be increased appropriately in the case of non-specific reactions. For complex templates, the annealing temperature needs to be adjusted to achieve efficient amplification.

4. Extension time setting: The extension time should be set according to the size of the amplified fragments. The following are recommended extension times:

Simple templates such as plasmids: 5-15 s/kb; Conventional genome, cDNA templates: 10-15 s/kb; Complex templates, crude extraction templates: 20-30 s/kb;

(The extension time should not be too short it should be at least 5 s/kb and not more than 30 s/kb).

5. Cycle number setting: 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.