2×Flash PCR MasterMix(Dye)

Catalog number:

F665667 (5 ml)

F665667 (40 ml)

Storage condition: -20℃

Product Content:

Component	5 ml	40 ml
2×Flash PCR MasterMix (Dye)	5 x 1 ml	40 x 1 ml
ddH2O	5 x 1 ml	40 x 1 ml

Products Introduction

This product is a premixed system consisting of a new high efficient fast DNA Polymerase, Mg2+, dNTPs, and PCR stabilizers and enhancers at 2× concentration. It is a new rapid DNA polymerase developed by CombiSigma with high amplification speed and stability. The extension speed is up to 5 s/kb, and the PCR can be completed in as little as 15 minutes, while longer fragments (>3 kb) or complex templates can be extended at a speed of 10-30 s/kb or a higher number of cycles. The unique MasterMix formula makes the whole reaction system very stable, while complex templates can be amplified effectively, and more than 98% of PCR amplification can be successful in one run. Simply add the DNA template and primers and top up with water to minimize human error, contamination and time.

The dye (blue) has been added to the product and it is ready for electrophoretic detection at the end of the reaction. The PCR product is amplified with an 'A' base at the 3′ end and can therefore be used directly for T/A cloning and is suitable for use in the CombiVerge Seamless Cloning Kit, T4 Ligation Kit and sensory products.

This product is mainly suitable for ultra-fast PCR, complex templates, complex secondary structures, gene cloning and large-scale genetic testing that requires high fidelity.

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.

Usage

The following is an example of a PCR reaction system and reaction conditions for amplifying a 1 kb fragment using 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.

PCR reaction system

reagents	50 μl system	25 μl system	20 μl system	final
2×Flash PCR MasterMix (Dye)	25 μl	12.5 μl	10 μl	1×
Forward Primer, 10 μM	2 μl	1 μl	0.8 μl	0.4 μM
Reverse Primer, 10 μM	2 μl	1 μl	0.8 μl	0.4 μM
Template DNA	<0.5 μg	<0.25 μg	<0.2 μg	<0.5 μg/50 μl
ddH2O	up to 50 μl	up to 25 μl	up to 20 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 concentration can be increased; if a non-specific reaction occurs, the primer concentration can be decreased to optimize the reaction system.

PCR reaction conditions

move	temp	timing	
precession	98°C	30 s1)	
sexual	94°C	10 s	30-35 cycles
annealing	55-65°C	15 s	30-35 cycles
reach	72°C	5 -15 s/kb	30-35 cycles
ultimate extension	72°C	1 min	

Note: 1) Note: For simple templates, the pre-denaturation time can be controlled at 30 s-1 min, for complex templates such as bacterial fluids, the pre-denaturation time can be increased to 2 min.

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 1. 30-35 number of cycles
2 . Primer concentration setting: The primer concentration can be set between 0.1 μM and 1.0 μM. A low 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 is 5℃ lower than the melting temperature of amplification primer Tm, so the annealing temperature can be lowered appropriately when the desired amplification efficiency cannot be obtained; the annealing temperature can be raised appropriately when non-specific reaction occurs. For complex templates, it is necessary to adjust the annealing temperature to achieve efficient amplification.
4. Extension time setting: The extension time should be set according to the size of the amplified fragments. The following extension times are recommended: simple templates such as plasmids: 5-15 s/kb; regular genomes, cDNA templates: 10-15 s/kb; complex templates, crude templates: 20-30 s/kb; (the extension time should not be too short and should be at least 5 s/kb, but should not exceed 30 s/kb).

5. Number of cycles: 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.