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2×HiFi PCR Mix for NGS

Product Number: NG01

Shipping and Storage

-20℃. For frequent use, store at 2-8℃.

Components

Component	NG01S 1mL	NG01M 5mL
2×HiFi PCR Mix	1mL	5×1mL
ddH_2O	1mL	5×1mL

Product Introduction:

The 2×HiFi PCR Mix for NGS is a premixed system composed of hot start enzymes, PCR Buffer, dNTPs, Mg^{2+}, as well as PCR stabilizers and enhancers. It has the characteristics of high fidelity, high elongation, and low bias, and has balanced amplification efficiency for complex DNA templates (such as high GC content templates), making it particularly suitable for the amplification of multiple PCR in second-generation library construction.

The high-efficiency hot start enzyme contained in this product has no polymerase activity at room temperature, effectively avoiding non-specific amplification caused by non-specific binding of primers and templates or primer dimers at room temperature. The combination of a unique buffer system and hot start enzymes significantly improves the amplification efficiency of PCR, with a wider amplification range.This product effectively improves the amplification efficiency of high GC or high AT regions in the genome, reduces amplification preference, and improves sequencing coverage.

Notes:

1. This product is not suitable for primers with modifications.
2. Before use, please gently mix it upside down to avoid foaming, and use after briefly centrifuging.
3. Avoid repeated freeze-thaw, as repeated freeze-thaw may cause a decrease in product performance. If frequent use is required in the short term, it can be stored at 2-8℃.

Protocol:

The following protocol is an example of conventional PCR reaction system and condition. The actual protocol should be improved and optimized based on the template, primer structure and the size of the target.

1. PCR reaction system:

Reagent	50μL
2×HiFi PCR Mix	25μl
Primer Pool	0.1-0.3μM
DNA or cfDNA	5ng-100ng
ddH_2O	up to 50μl

Note: Please use the final concentration of 0.1-0.3μM as a reference for setting the primer concentration range.

2. PCR reaction program:

Step	Temperature	Time	
Pre-denaturation	95℃	2min	
Denaturation	98℃	20s	35-40cycles
Annealing/Extension	65℃	90 s	
Final extension	72℃	5min	

Note:1)In general experiments, the annealing temperature is 5℃ lower than the melting temperature Tm of the amplification primer, and when ideal amplification efficiency cannot be achieved, the annealing temperature should be appropriately reduced; When a non-specific reaction occurs, increase the annealing temperature to optimize the reaction conditions.

2)The number of cycles can be set based on the downstream application of the amplification product. If the number of cycles is too small, the amplification amount is insufficient: If the number of cycles is too many, the probability of mismatch will increase, and the non-specific background will be severe. So, while ensuring product yield, the number of cycles should be minimized as much as possible.