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LiTaq™ Ultra HiFi DNA Polymerase

Cat. #: M0030 Size: 100/500 U

Introduction

LiTaq™ Ultra HiFi DNA Polymerase is a fast and super-fidelity DNA Polymerase with high amplification efficiency. The Polymerase possesses 5 '-3' DNA Polymerase activity and 3 '-5' exonuclease activity. The enzyme was modified by other high-fidelity enzymes with strong amplification ability, fast amplification speed and high fidelity, which overcame the defects of ordinary Pfu enzyme such as poor amplification ability, low yield and slow amplification speed, and greatly shortened the reaction time. This product can be used for long fragment amplification and the expansion of other complex templates. The 3 'end of the amplified PCR product does not contain "A" base, and can be directly cloned in the flat terminal vector. If T/A cloning is needed, "A" should be added to the end of the PCR product for cloning. This product is suitable for gene cloning, gene point mutation, SNP amplification experiments.

Package Information

Components	M0030-01 (100 U)	M0030-01 (500 U)
LiTaq™ Ultra HiFi DNA Polymerase (2 U/μl)	50 μl	250 μl
2× Ultra HiFi Buffer	2×1.25 ml	7×1.8 ml
dNTP Mix, 10 mM each	150 μl	750 μl

Storage

All materials should be stored at -20°C.

Recommended PCR Reaction System

2× Ultra HiFi Buffer	25 μl	1×
dNTP Mix, 10 mM each	1.5-2.5 μl	300-500 μM each
Template DNA	Appropriate	<500 ng/50 μl
Forward Primer, 10 uM	2 μl	0.4 μM
Reverse Primer, 10 uM)	2 μl	0.4 μM
LiTaq™ Ultra HiFi DNA Polymerase	0.5-0.75 μl	1-1.5 U/50 μl
ddH_2O	to 50 μl	

Note:

All operations should be carried out on ice. After thawing, mix the components horoughly. After use, please put them back to -20°C in time.

FOR RESEARCH USE ONLY

Recommended PCR Reaction Program

Steps	Temperature	Time	Cycles
Initialization	98°C	30 sec-3 min	1
Denaturation	98°C	10-30 sec	25-35
Annealing	According to Tm	15-30 sec	
Extension	72°C	3-5 kb/min	
Final Extension	72°C	5 mins	1

Note:

1) The three-step amplification method is preferred. The three-step method cannot amplify the target product or primer with Tm value greater than 68°C, so please try the two-step method.

2) Denaturation: the pre-denaturation of simple templates is 98°C, 30s to 1min, and the pre-denaturation time can be extended to 3min for complex templates.

3) Annealing: in general experiments, the annealing temperature is 3-5°C lower than the Tm value of the primer. If the ideal amplification efficiency cannot be obtained, the annealing temperature should be changed gradient for optimization; When non-specific reaction occurs, the annealing temperature should be increased appropriately.

4) Extension: The extension time should be set according to the length of the amplified fragment and the complexity of the template. The amplification efficiency of this product is 3-5 kb /min, and 2-4 kb/min is recommended for long fragments and templates with high complexity.

5) Cycle number: the cycle number can be set according to the downstream application of the amplification product. If the cycle number is too small, the expansion increment is insufficient, and the cycle number is too large, the mismatch probability will increase. Therefore, the cycle number should be reduced as far as possible on the premise of ensuring the yield of the product.as possible yet ensuring the yield of the product.

Primers Designing Notes

1. Choose C or G as the last base of the 3'-end of the primer.
2. Avoid continuous mismatching at the last 8 bases of the 3'-end of the primer.
3. Avoid hairpin structure at the 3'-end of the primer.
4. T_m of the primers should be within the range of 55°C-65°C.
5. Additional sequence should not be included when calculating T_m of the primers.
6. GC content of the primers should be within the range of 40% - 60%.
7. T_m and GC content of forward and reverse primes should be as similar as possible.