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2× LiTaq™ Plus PCR Master Mix

Cat. #: M0025 Size: 5 ml/15 ml/50 ml

Introduction

2× LiTaq™ Plus PCR Master Mix is a mixture of LiTaq™ Plus DNA polymerase, dNTP, an optimized buffer system and an enzyme containing 3'→5' exonuclease activity. Its fidelity is 6 times greater than that of LiTaq™ DNA Polymerase. Compared with LiTaq™ DNA Polymerase, LiTaq™ Plus DNA polymerase has stronger amplification performance, higher sensitivity, and is more tolerant of impurities within 5 kb amplifying range. It can amplify up to 10 kb from human genomic DNA or up to 15 kb from λ DNA. Protective agents in the 2× LiTaq™ Plus Master Mix enable the resistance to repeated freeze-thaw cycles. The obtained PCR products are compatible with LiClone™ Ultra One Step Cloning Kit (Cat. #: M0011), and can be directly used for cloning into T-Vectors as most PCR products amplified with Taq DNA polymerase have one A at the 3'-terminus.

Package Information

Components	M0025-05	M0025-15	M0025-50
2× LiTaq™ Plus PCR Master Mix	5×1 ml	15×1 ml	50×1 ml

Storage

All materials should be stored at -20°C.

Quality Control

Exonuclease Activity: A reaction containing 10 U of enzyme and 0.6 μg of λ-Hind III incubated for 16 hours at 37°C resulted in no visually discernible change to DNA as determined by agarose gel electrophoresis.

Endonuclease Activity: A reaction containing 10 U of enzyme and 0.6 μg of Supercoiled pBR322 DNA incubated for 4 hours at 37°C resulted in no visually discernible conversion to nicked circular DNA as determined by agarose gel electrophoresis.

Functional Assay: 30 cycles of PCR amplification of 100 ng human genomic DNA with 1.25 units of LiTaq™ DNA Polymerase results in the expected 360 bp α-1-antitrypsin gene product, as determined by agarose gel electrophoresis.

Protocol

1. General reaction mixture for PCR:

Components	Reaction Volume
2× LiTaq™ Plus PCR Master Mix	25 μl
Template DNA*	Optional
Primer 1 (10 μM)	2 μl
Primer 2 (10 μM)	2 μl
ddH_2O	to 50 μl

* The recommended amount of DNA template for a 50 μl reaction is as follows:

Human Genomic DNA	0.1~1 μg
Bacterial Genomic DNA	10~100 ng
λDNA	0.5~5 ng
Plasmid DNA	0.1~10 ng

2. Thermocycling Conditions for a routine PCR:

Step	Temperature	Time	Cycle
Initialization	94°C	5 min	1
Denaturation	94°C	15 sec	35
Annealing	55°C*	30 sec	
Extension	72°C	60 sec/kb	
Final Extension	72°C	7 min	1
Holding	4°C	-	1

* Annealing temperature is based on the Tm of the primer pair and is typically 1-2°C below the calculated Tm.

Handling Notes

LiTaq™ Plus DNA Polymerase also shows polymerase activity at room temperature. Thus, it is better to set up reaction systems on ice and immediately start the reaction when it is done, so as to reduce nonspecific amplification in the preparatory stage and get better PCR result.

Primers Designing Notes

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

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