

## Introduction

**2× LiTaq™ PCR Master Mix** is an optimized ready-to-use solution containing LiTaq™ DNA polymerase, dNTPs, MgCl<sub>2</sub>, KCl and stabilizers. 2× LiTaq™ Master Mix are stable for 20 freeze-thaw cycles when stored at -20°C. The presence of two tracking dyes allows direct loading of the PCR product onto agarose gels. The obtained PCR products are compatible with **LiClone™ One Step Cloning Kit** (Cat. #: M0010), 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	M0024-05	M0024-15	M0024-50
2× LiTaq™ 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:

2× LiTaq™ PCR Master Mix	25 µl
Template DNA*	Optional
Primer 1 (10 µM)	2 µl
Primer 2 (10 µM)	2 µl
ddH <sub>2</sub> O	to 50 µl

## 2× LiTaq™ PCR Master Mix

Cat. #: M0024 Size: 5 ml/15 ml/50 ml

\* 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:

94°C	5 min (Pre-denaturation)	
94°C	15 sec	} 35 cycles
55°C*	30 sec	
72°C	60 sec/kb	
72°C	7 min (final extension)	
4°C	Hold	

\* Annealing temperature is based on the T<sub>m</sub> of the primer pair and is typically 1-2°C below the calculated T<sub>m</sub>.

## Handling Notes

LiTaq™ 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. T<sub>m</sub> of the primers should be between 55°C~65°C;
5. 5' adding sequence should not be included when calculating T<sub>m</sub> of the primers;
6. GC content of the primers should be between 40%~60%;
7. T<sub>m</sub> and GC content of forward and reverse primers should be as similar as possible.