LIFE SCB
LIFE SCIENCE BIOTECHNOLOGY

Email: info@lifescb.com
Tel: +1-301-328-1977 Fax: +1-301-965-8269
9610 Medical Center Drive, Rockville, MD 20850, USA

LiTaq™ DNA Polymerase (Mg2+ plus buffer)

Cat. #: M0023 Size: 1000 U/10000 U

Introduction

LiTaq™ DNA Polymerase is a thermostable DNA polymerase that exhibits a 5' → 3' polymerase activity and a 5' → 3' exonuclease activity, with no 3' → 5' exonuclease activity. Taq DNA Polymerase is purifed from an *Escherichia coli* (*E.coli*) strain overexpressing the gene of Thermus aquaticus DNA Polymerase. No endonuclease, exonuclease, or bacterial DNA were detected in this kit. The PCR products contain A at the 3'-end and can be directly cloned into T-Vectors. The products are also compatible with LiClone™ Ultra One Step Cloning Kit (Cat. #: M0011).

Package Information

Components	M0023-01 (1000 U)	M0023-10 (10000 U)
LiTaq™ DNA Polymerase (5 U/μl)	200 μl	2000 μl
10× Taq Buffer (Mg^{2+} plus)	4 ml	40 ml

Storage

All materials should be stored at -20°C.

Unit Definition

One unit (U) is defined as the amount of enzyme that incorporates 10 nmol of dNTPs into acid-insoluble products in 30 minutes at 74°C, with activated salmon sperm DNA as the template/primer.

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:

LiTaq™ DNA Polymerase (5 U/μl)*	0.5 μl
10× Taq Buffer (Mg^{2+} plus)	5 μl
25 mM $MgCl_2$**	Optional
dNTP Mix (10 mM each)	1 μl
Template DNA***	Optional
Primer 1 (10 μM)	2 μl
Primer 2 (10 μM)	2 μl
ddH_2O	to 50 μl

* The amount of Taq DNA Polymerase can be adjusted between 0.25 μl and 1 μl. Generally, higher level of Taq will increase the yield of PCR products but may decrease the specificity of PCR amplifcation.

** The final concentration of Mg2+ of this mixture is 2 mM, as for most PCR reactions, the optimized final concentration of Mg2+ is 1.5 - 2 mM. However, if necessary, the concentration of Mg2+ can be increased by adding 25 mM MgCl .

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

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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