AceTaq DNA Polymerase

Catalog# P401

Version 9.1

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Introduction

AceTaq DNA Polymerase is a chemically modified Taq DNA Polymerase that is completely blocked at room temperature and is released only after heating at 95°C. Non-specific amplification and primer dimerization can be prevented during sample preparation and reaction ramps. Compared with the antibody-based hot-start Taq, the polymerase activity of AceTaq is blocked more stringently and completely. It takes only 5 min to activate AceTaq. AceTaq is compatible with most existing PCR protocols. Combined with an optimized buffer system, AceTaq minimizes non-specific amplification and primer dimers, ensuring extremely high sensitivity and specificity, which makie it ideal for amplifying low-copy genes from complex templates. The 3'-end of the PCR products contain A, which can be directly cloned to a T-vector and is suitable for ClonExpress and Topo Cloning Kits (#C112 / #C113 / #C115 / #C601).

Components

Components	P401-d1	P401-d2	P401-d3
10 × AceTaq Buffer (Mg^{2+} Plus)	1 ml	4 × 1 ml	
dNTP Mix (10 mM each)	200 µl	800 µl	3 × P401-d2
AceTaq DNA Polymerase (5 U / µl)	50 µl	200 µl	

Storage

Store at -30°C ~ -15°C. Transport at -20°C ~ 0°C.

Unit Definition

One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid-insoluble material in 30 min at 74℃, using activated salmon sperm DNA as template/primer.

Protocol

Reaction system

ddH_2O	to 50 µl
10 × AceTaq Buffer (Mg^{2+} plus)	5 µl
dNTP Mix (10 mM each)	1 µl
Template DNA*	x µl
Primer 1 (10 µM)	2 µl
Primer 2 (10 µM)	2 µl
AceTaq DNA Polymerase (5 U / µl) **	0.5 µl

* The optimal concentration is different for different templates. The following table shows the recommended template usage for a 50-µl reaction system:

Human genomic DNA	1-500 ng
E. coli genomic DNA	1-100 ng
λ DNA	0.1-1 ng
Plasmid DNA	0.1-1 ng

** The amount of AceTaq can be adjusted between 0.25 µl-1 µl. Increasing the amount of enzyme may increase the yield of amplification, but it may also reduce the specificity.

Reaction Program

95℃	5 min * (pre-denaturation)	
95℃	30 sec	30 -35 cycles
55℃ **	30 sec	
72℃	60 sec / kb	
72℃	7 min (complete extension)	

* The pre-denaturation takes at least 5 min. If the amplification is not ideal, extend the pre-denaturation up to 10 min.

** The annealing temperature needs to be adjusted according to the Tm value of the primer (normally set as be 3°C - 5°C lower than the primer Tm).

Tips for Primer Design

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. The Tm values of the forward primer and the reverse primer are preferably not more than 1°C, and the Tm value is preferably adjusted to 55°C-65°C. (Primer Premier 5 is recommended to calculate Tm value).

5. Primer additional sequence, i.e., unpaired sequence with template, should not be calculated for Tm value.

6. GC content of the primers should be between 40% - 60%.

7. The overall distribution of primers A, G, C, and T should be as uniform as possible, avoiding the use of high GC/AT content region.

8. Avoid a complementary sequence of more than 5 bases inside of the primer or between the two primers. Avoid a complementary sequence of more than 3 bases at the 3'-end of the two primers.

9. Use the NCBI BLAST function to search for primer specificity to avoid non-specific amplification.