Taq DNA Polymerase DNA-free

From Thermus aquaticus BM, recombinant (E.coli)

Deoxynucleoside-triphosphate: DNA deoxynucleotidyltransferase, Taq Pol, EC 2.7.7.7
Product code A5434

Stability: 12 months under proper storage conditions Storage: -20°C

Description

AppliChem's *Taq DNA Polymerase (DNA-free)* A5434 is a genetically engineered, DNA-free thermostable DNA polymerase originally isolated from Thermus sp. A5434 Taq DNA Polymerase is especially useful for 16S + 23S rDNA gene amplification and cloning, because the enzyme preparation is free of genomic and plasmid DNA from the production strain. The enzyme has the following activities: 5'-3' polymerase (60 to 150 nucleotides/s; approx. 1 kb/min), 5'-3' exonuclease (strand displacement) and 3' terminal deoxynucleotidyl transferase (addition of single dATP to the duplex DNA). Taq DNA Polymerase incorporates modified nucleotides (dNTPαS, c7GTP, biotin-11-dUTP, digoxigenin-11-dUTP and fluorescein-12-dUTP but not biotin-16-dUTP) at high rates. The error frequencies for misincorporation and frameshift mutations are 10-4 and 2 x 10-5, respectively. Only the last three bases adjacent to the 3' end of the primer need to be correctly base-paired in order to initiate polymerization. The 5' region of the primer is less sensitive to base mismatches. Thus, new restriction sites can be easily introduced into an amplification product.
Up to 9 kb can be amplified from lambda DNA and up to 5 kb from genomic DNA.

Unit definition: One unit of enzyme incorporates 10 nmoles of dNTPs into an acid-insoluble form in 30 min at 72 °C under standard assay conditions, using a DNA template. Specific activity is 50.000 U/mg protein.

Kit Components

Component	A5434,0100	A5434,0500
Taq DNA Polymerase	100 U	500 U
PCR Buffer 10X (1.5 mM $MgCl_2$ final concentration)	1 x 1.5 ml	2 x 1.5 ml
PCR Water	2 x 1.25 ml	10 x 1.25 ml

Quality control

All of AppliChem's DNA-free reagents are tested for absence of bacterial DNA (at least 40 cycles of qPCR).
Each lot of A5434 Taq DNA Polymerase (DNA-free) is tested for performance in PCR runs, using different primer pairs for amplification of sequences between 0.5 and 3 kb.

Applications

- Polymerase chain reaction (PCR)
- 16S and 23S rRNA gene amplification (without background)
- detection of bacteria in samples (e.g. blood)
- DNA labeling reactions
- Sequencing/ cycle sequencing

Enzyme Properties

Volume activity	5 U/µl
Error rate*	approx. 1.3x10-5
Optimal enzyme concentration	0.5-2.5 U per 50 µl reaction
Standard enzyme concentration	1.25 U per 50 µl reaction
Optimal pH	approx. 9 (adjusted at 20°C)
Optimal polymerization temperature	approx. 72°C
Optimal Mg^{2+} concentration	1.5 – 5 mM
Standard Mg^{2+} concentration	1.5 mM when using 200 µM dNTP each PCR product size optimized for up to 5 kb
PCR cloning	TA-cloning; addition of A-overhangs ("extendase activity")
Incorporation of modified nucleotides	accepts modified nucleotides like radiolabeled nucleotides, DIG-dUTP, biotindUTP
Thermostability	> 80% activity after 30 cycles (1 min 95°C, 1 min 37°C, 3 min 72°C)

* according to the lacI assay (Frey, B. and Suppmann, B. (1995) *Biochemica* **2**, 8-9.)

Standard PCR procedure

General considerations

The optimal conditions (incubation times and temperatures, concentrations of enzyme, template DNA, Mg^{2+}) depend on the system used and have to be determined individually. In particular, the Mg^{2+} concentration and the amount of enzyme used per assay should be titrated for optimal efficiency of DNA synthesis. As a starting point, use the following guidelines:

- Optimal enzyme concentration: 0.5 – 2.5 U/50 µl reaction.
- Optimal Mg^{2+} concentration can vary from 1.5 mM to 5 mM; in most cases a Mg^{2+} concentration of 1.5 mM will produce satisfactory results when using 200 µM dNTP (each). The 10x PCR buffer supplied contains 1.5 mM Mg^{2+}.
- dNTP concentration: always use balanced solutions of all four dNTP. The final concentration of each dNTP should be between 50 and 500 µM; the most commonly used concentration is 200 µM. Increase concentrations of Mg^{2+} when increasing the concentration of dNTP.
- Template concentration: e.g. human genomic DNA template: 10 ng-250 ng; plasmid DNA template: 0.1 ng-15 ng.
- The optimal dilution buffer for the template DNA is either simply sterile double-distilled water or 5-10 mM Tris (pH 8-9). Storage of DNA should be in 10 mM Tris pH 8.0-8.5 or TE buffer (10 mM Tris pH 8.0; 1 mM EDTA).

Preparation of reaction mixtures

Briefly centrifuge all reagents before starting. Keep the *Taq DNA Polymerase* vial chilled. Place the *Taq DNA Polymerase* vial back to the freezer immediately after handling.
Prepare a mix of reagents in sterile thin-walled PCR tubes on ice:

Reagents	**Volume**	**Final concentration**
Sterile ddH_20	add up to 50 µl	
10x PCR reaction buffer	5 µl	1x (1.5 mM Mg^{2+})
dNTP mix (10 mM)	1 µl	200 µM (of each dNTP)
Primer 1	variable	0.1-0.6 mM
Primer 2	variable	0.1-0.6 mM
Template DNA[§]	variable	0.1-1000 ng
Taq DNA Polymerase (5 U/µl)	0.4 µl 2 U/ reaction	
Final volume	**50 µl**	

For more reactions, prepare a master mix containing sterile ddH2O, 10 x PCR reaction buffer, dNTP mix (10 mM) and *Taq DNA Polymerase* (5 U/µl) in proportions as indicated in the table above. Please note that the volume of ddH_2O added is corrected for the volumes of primer 1 and 2 and template DNA. Pipette aliquots of the master mix into PCR tubes and add primers and template DNA.
Gently vortex the mixture to produce a homogenous reaction, then centrifuge briefly to collect the sample at the bottom of the tube. Continue to thermal cycling immediately.
Note: Carefully overlay the reaction with mineral oil if required by your type of thermal cycler.

[§] For genomic DNA template 10 ng – 1000 ng; for plasmid DNA 0.1 ng – 15 ng

Thermal cycling

Place the samples in the thermal cycler, and start cycling. For single extension time use the protocol below:

	Temperature	**Time**	**Cycle No.**
Initial denaturation*	94°C	4 min	1x
Denaturation*	94°C	15-30 sec	25-40x
Annealing#	45-65°C	30-60 sec	
Elongation+	72°C	45 sec - 3min	
Final elongation	72°C	10 min	1x
Cooling	4°C	unlimited time	

[*] The denaturation temperature can vary from 92°C-95°C.
[#] Optimal annealing temperature depends on the melting temperature of the primers and on the system used.
[+] For PCR products up to 1 kb elongation temperature should be around 72°C; for PCR products larger than 1 kb elongation temperature should be around 68°C.

Check PCR products by agarose gel electrophoresis. (After cycling, samples may be frozen for storage).