Taq DNA Polymerase recombinant, 5 U/µl

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
AP0100104	2000 U	*Taq* DNA Polymerase recombinant (1 × 400 µl) 10× Reaction Buffer (2 × 1.5 ml) 50 mM $MgCl_2$ (1 × 1.5 ml)

COMPONENT	COMPOSITION
Taq DNA Polymerase, Recombinant, 5 U/µl	*Taq* DNA Polymerase, 5 U/µl, in storage buffer containing 50% (v/v) glycerol
10× Reaction Buffer	Optimized PCR buffer without magnesium ions
50 mM $MgCl_2$	50 mM $MgCl_2$ in water
STORAGE	–20°C until expired
SHIPMENT	Cool pack

FEATURES

- High product yields and robustness in a wide application range
- Highest quality utilized in molecular diagnostics and research
- Exceptionally pure *Taq* DNA Polymerase for routine and demanding PCR applications

APPLICATIONS

- Routine and applied PCR up to 5 kb
- RT-PCR
- TA cloning

DESCRIPTION

Apsalagen™ *Taq* DNA polymerase is a first-choice enzyme for all routine and molecular diagnostics PCR applications. The exceptional quality and purity of the enzyme ensures the highest performance that is utilized by the diagnostic industry and research labs. The polymerase is suitable for standard and fast PCR applications giving high product yields from various templates with targets of up to 5 kb in size.
Taq DNA polymerase is a thermostable, highly processive 5'→3' DNA polymerase that has low 5'→ 3' exonuclease activity and lacks 3'→5' exonuclease (proofreading) activity. The latter allows incorporation of modified nucleotides.
The enzyme also exhibits deoxynucleotidyl transferase activity that results in the addition of extra A overhang at the 3'-ends of PCR products, allowing easy cloning of PCR products into vectors with T overhangs.

PROTOCOL

Prevention of PCR contamination

When assembling the amplification reactions, care should be taken to eliminate the possibility of contamination with undesired DNA.

- Use separate clean areas for preparation of samples and reaction mixtures and for cycling.
- Wear fresh gloves. Use sterile tubes and pipette tips with aerosol filters for PCR setup.
- Use only water and reagents that are free of DNA and nucleases.
- With every PCR setup, perform a contamination control reaction that does not include template DNA.

Standard PCR setup

The standard PCR protocol using reaction buffer provides excellent results for most applications. Optimization might be necessary for certain conditions, such as high GC or AT content, strong template secondary structures or insufficient template purity. In such cases, optimization of template purification, primer design and annealing temperature is recommended.
The best conditions for each primer-template can be optimized with the following:

- Choosing the optimal quantities of template and primers
- Determining optimal concentrations of the enzyme and magnesium ions
- Optimizing cycling conditions

If unspecific amplification occurs, the amount of *Taq* DNA Polymerase and the primer concentration can be reduced. Correspondingly, these can be increased when yield is low.

Optimizing magnesium concentration

Many applications use the standard concentration of 1.5 mM $MgCl_2$. However, reactions with increased amounts of template (e.g., genomic DNA), primer and nucleotides might require higher $MgCl_2$ concentrations (2–3 mM). A separate 50 mM $MgCl_2$ solution is supplied with the enzyme and can be used to adjust the $MgCl_2$ concentration according to the table below:

Final concentration of $MgCl_2$ in a 50 µl reaction, mM	1.5	1.75	2.0	2.5	3.0
Volume of 50 mM $MgCl_2$ solution to add, µl	1.5	1.75	2.0	2.5	3.0

BASIC PROTOCOL

- Thaw on ice and mix all reagents well, especially the $MgCl_2$ solution and dNTPs.
- Keep all reagents and reactions on ice.
- When setting up multiple reactions, prepare a master mix of water, buffer, dNTPs and polymerase. Prepare enough master mix for one more than the actual number reactions. Alternatively, use PCR Master Mix, 2×
- Pipet the master mix into thin-walled 0.2 ml PCR tubes.
- Add template and primers separately if they are not used in all reactions.

COMPONENT	VOLUME	FINAL CONCENTRATION
10× Reaction Buffer	5 µl	1×
50 mM $MgCl_2$	Variable (standard 1.5 µl)	1.5–3 mM
	Higher than 2 mM $MgCl_2$ might increase yield but reduce fidelity	
10 mM dNTP Mix	1 µl	200 µM
Forward primer	Variable	0.2–1 µM
Reverse primer	Variable	0.2–1 µM
Template DNA	Variable	10 pg – 1 µg
	Use 0.01–1 ng for plasmid or phage DNA and 0.1–1µg for genomic DNA	
Taq DNA Polymerase (5 U/µl)	0.5 -1.0 µl	2.5 - 5 U
Nuclease free water	Variable	
Total volume	50 µl	

- Mix and centrifuge briefly to collect the liquid in the bottom of the tube.
- Place in the PCR cycler.

CYCLING PROGRAM

STEP	TEMPERATURE	TIME	CYCLES
Initial activation	94°C	3–5 min	1
Denaturation	94°C	30 s	25–35
Annealing	55°C	15–30 s	25–35
	Approximately 5°C below T_m of primers		
Extension	72°C	30–60 s/kb	25–35
Final extension	72°C	5 min	1
	To extend all incomplete PCR products		
Storage in the cycler	4°C	Indefinitely	1

- Add loading dye solution to the reactions to analyze PCR products on a gel or store them at –20°C.

CERTIFICATE OF ANALYSIS

Functional assay
Human genomic DNA (100 ng) was amplified using Taq DNA Polymerase and specific primers to produce a distinct band of 750 bp.

Self-priming activity
Standard PCR is carried out without primers, using Taq DNA Polymerase and human genomic DNA. No products were amplified.

Exonuclease assay
Linearized lambda/HindIII fragments (1 µg) are incubated with 10 U Taq DNA Polymerase in a 50 µl reaction mixture for 4 h at 37°C. No degradation of DNA was observed.

Endonuclease assay
lambda DNA (1 µg) is incubated with 10 U of Taq DNA Polymerase in a 50 µl reaction mixture for 4 h at 37°C. No degradation of DNA was observed.

Nick assay
Supercoiled plasmid DNA (1 µg) is incubated with 10 U Taq DNA Polymerase in a 50 µl reaction mixture for 4 h at 37°C. No conversion of covalently closed circular DNA to nicked DNA was detected.

E. coli genomic DNA contamination assay
A sample of 5 µl denatured Taq DNA Polymerase is analyzed with specific primers for the 16S rRNA gene in qPCR for the presence of contaminating *E. coli* genomic DNA. The detection limit is < 1 copy genome per unit Taq DNA Polymerase. No genomic DNA was detectable.

Unit Definition
One unit is defined as the amount of Taq DNA Polymerase required to catalyze the incorporation of 10 nmol dNTP into acid-insoluble form in 30 min at 72°C in the presence of the reaction buffer.

USEFUL HINTS

- Visit Support at www.apsalagen.com for product selection guides.
- Visit Applications at www.apsalagen.com for more nucleic acid purification and analysis products.
- Visit OEM at www.apsalagen.com for custom product formulations and bulk amounts.

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