ApStarTaq™ DNA Polymerase

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR0201501	250 U	50 µl ApStarTaq DNA Polymerase, 5 U/µl 1.8 ml 5× Reaction Buffer 1.5 ml 50 mM $MgCl_2$
BR0201502	1250 U	250 µl ApStarTaq DNA Polymerase, 5 U/µl 3 × 1.8 ml 5× Reaction Buffer 1.5 ml 50 mM $MgCl_2$

COMPONENT	COMPOSITION
ApStarTaq DNA Polymerase	ApStarTaq DNA Polymerase, 5 U/µl, in storage buffer containing 50% (v/v) glycerol
5× Reaction Buffer	Optimized PCR reaction buffer without magnesium ions
50 mM $MgCl_2$	50 mM $MgCl_2$ in water
STORAGE	–20°C (until expiry date – see product label)

FEATURES

- Aptamer-based hot-start functionality
- No activation steps needed
- High PCR specificity and sensitivity
- Exceptionally pure Taq DNA Polymerase for sensitive PCR applications and high yields

APPLICATIONS

- Fast PCR reactions in endpoint and real-time analysis
- Hot-start PCR up to 3 kb
- Amplification of low-copy-number targets
- RT-PCR and TA cloning

DESCRIPTION

biotechrabbit™ ApStarTaq DNA Polymerase is an aptamer based hot-start enzyme and the first-choice for fast PCR reactions. The exceptional quality and purity of the enzyme ensures highest performance, suitable for standard and fast PCR cycling in both, endpoint and real-time assays. It ensures high product yields with low background and without primer–dimer formation or non-specific priming.
The aptamer binds to *Taq* DNA Polymerase and inhibits the enzyme activity at temperatures below 45°C. This ensures full hot-start functionality. The enzyme is released during standard PCR cycling conditions. There is no need for separate heating or denaturation steps, allowing fast PCR reactions.

Info: Recommended annealing temperature is 2°C above primer Tm (use gradient PCR to optimize the annealing temperature).

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 biotechrabbit 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 (see biotechrabbit nucleic acid purification kits), 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 ApStarTaq DNA Polymerase and the primer concentration can be reduced. Correspondingly, these can be increased when yield is low.

Optimizing magnesium concentration

Typically, the standard concentration of 2.0 mM $MgCl_2$ is used. However, aptamer-based hot-start polymerase and/or reactions with increased amounts of template (e.g., genomic DNA), primer and nucleotides might require higher $MgCl_2$ concentrations. 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 $MgCl_2$ concentration in a 25 µl reaction, mM	2.0	2.5	3.0	3.5
Volume of 50 mM $MgCl_2$ solution to add, µl	1.0	1.25	1.5	1,75

BASIC PROTOCOL

- Thaw on ice and mix all reagents well, especially $MgCl_2$ and dNTPs. Keep all 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.
- 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
5× Reaction Buffer	5 µl	1×
50 mM $MgCl_2$	1.0 µl	2 mM
Do not use less than 2 mM $MgCl_2$. Higher than 2 mM might increase yield but reduce fidelity.		
10 mM dNTP Mix (BR0600202)	0.5 µl	200 µM
Forward/Reverse primer	Variable	0.2–1 µM each
Template DNA	Variable	10 pg–1 µg
Use 0.01–1 ng for plasmid or phage DNA and 0.1–1 µg for genomic DNA.		
ApStarTaq DNA Polymerase (5 U/µl)	0.25	1.25 U
Nuclease free water	Variable	
Total volume	25 µl	
For total reaction volumes other than 25 µl, scale reagents proportionally.		

- 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 DNA denaturation	95°C	1 min*	1
	** recommended time for denaturation of genomic DNA templates*		
Denaturation	95°C	30 s	25–35
Annealing*	(55-68°C)	15–30 s	25–35
**Recommended annealing temperature is 2°C above Tm of primers, or use gradient PCR to optimize the annealing temperature*			
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 (see DNA Loading Dye, 6×, BR0800301) to the reactions to analyze PCR products on a gel or store them at −20°C.

CERTIFICATE OF ANALYSIS

Unit Definition

One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nmol of dNTP into acid-insoluble form in 30 minutes at 72°C in the presence of the reaction buffer.

Quality Control

Functional assay

Human genomic DNA was amplified using the DNA Polymerase and specific primers to produce a distinct band of 750 bp.

Self-priming activity

Standard PCR is carried out without primers, using the DNA Polymerase and human genomic DNA. No products were amplified.

Exonuclease assay

Linearized lambda/HindII fragments are incubated with the DNA Polymerase in a 50 µl reaction mixture for 4 h at 37°C. No degradation of DNA was observed.

Endonuclease assay

lambda DNA is incubated with the DNA Polymerase in a 50 µl reaction mixture for 4 h at 37°C. No degradation of DNA was observed.

Nick Activity

Supercoiled plasmid DNA is incubated with the 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 DNA contamination assay

A sample of the denatured DNA Polymerase is analyzed with specific primers targeting the 16S rRNA gene in qPCR for the presence of contaminating *E. coli* DNA. No *E. coli* DNA was detectable.

Quality confirmed by: Head of Quality Control

SAFETY INSTRUCTIONS

For safety instructions please see Safety Data Sheets (SDS)/Sicherheitshinweise finden Sie in den SDS unter: http://www.biotechrabbit.com/support/documentation.html.

USEFUL HINTS

- Visit Applications at www.biotechrabbit.com for more products and product selection guides.
- Most biotechrabbit products are available in custom formulations and bulk amounts.

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Certified Quality-System mdc ISO 13485 ISO 9001

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