UPstart™ *Taq* Antibody, 1 mg/ml

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR1200203	1000 µg (5000 U)	1000 µl UPstart *Taq* Antibody

COMPONENT	COMPOSITION
UPstart *Taq* Antibody	UPstart *Taq* Antibody, 1 mg/ml (5 U/µl) in storage buffer containing 50% (v/v) glycerol
STORAGE	–20°C (until expiry date – see product label)

FEATURES

- Inhibition of >95% Taq activity at 60°C
- 200 ng UPstart Taq Antibody are blocking 1 U *Taq* DNA polymerase
- Exceptionally pure — made in cell culture

APPLICATIONS

- Thermolabile inhibition of *Taq* DNA polymerases
- Convenient hot-start PCR setup at room temperature
- Fast polymerase activation with the first PCR denaturation step

UPstart™ *Taq* Antibody, 1 mg/ml

DESCRIPTION

biotechrabbit™ Upstart *Taq* Antibody is an ultra-pure monoclonal antibody against the *Taq* DNA polymerase. It is produced in cell culture to ensure highest quality.
The antibody can be used with highly efficient *Taq* DNA polymerases, provides an excellent method for "hot start" PCR and enhances PCR specificity and sensitivity. PCR hot start prevents the formation of primer–dimers and nonspecific amplification and allows convenient PCR setup at room temperature.
In the first denaturation step of the thermal cycling, the UPstart *Taq* Antibody becomes nonfunctional and the active *Taq* DNA polymerase is released. This antibody-mediated hot-start method is significantly more convenient to use than other hot-start methods. Polymerase reactivation using this antibody is faster than with methods using chemically inhibited polymerases.

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.

Pre-mix preparation of *Taq* DNA polymerase and UPstart *Taq* Antibody

A pre-mix will result in a hot-start *Taq* DNA polymerase that can be used for the setup of hot-start PCR reactions. An example for a pre-mix is given below.

COMPONENT	VOLUME	FINAL CONCENTRATION
Taq DNA Polymerase, 5 U/µl (i.e. BR0100101)	20 µl	100 U
UPstart *Taq* Antibody	20 µl	100 U
Gives 100 reactions with 1 unit Hot-start Taq DNA polymerase per reaction.		
For one reaction use:		
Taq DNA Polymerase / UPstart *Taq* Antibody pre-mix	0.4 µl	1 U Hot-Start *Taq* DNA Polymerase

- Mix and centrifuge briefly to collect the liquid in the bottom of the tube.
- The mix can be stored at -20°C for up to 6 months.

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, UPstart *Taq* antibody and *Taq* DNA polymerase. Prepare enough master mix for one more than the actual number reactions. Alternatively, use biotechrabbit Hot-Start PCR Master Mix, 2× (cat. no. BR0200201)
- 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
Taq DNA Polymerase, 5 U/µl	0.2 µl	1 U
UPstart *Taq* Antibody	0.2 µl	1 U
Mix Taq DNA polymerase and UPstart Taq Antibody and incubate at room temperature for 15 min. Alternatively use pre-mixed Taq DNA polymerase and UPstart Taq Antibody.		
10× Reaction Buffer	5 µl	1×
50 mM $MgCl_2$	Variable (standard 1.5 µl)	1.5 mM
	Higher than 2 mM $MgCl_2$ might increase yield but reduce fidelity	
5× PCR Enhancer (optional)	10 µl	1×
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 0.1–1 µg for genomic DNA	
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 (see DNA Loading Dye, 6×, cat. no. 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 antibody required to inhibit >95% of 1 unit *Taq* DNA polymerase at 60°C. One unit UPstart *Taq* Antibody (200 ng of the antibody) will ensure inhibition of *Taq* DNA polymerase.

Quality Control

Functional Assay

UPstart *Taq* Antibody was functionally tested on hot-start PCR.

Inhibition activity

The *Taq* DNA polymerase activity was inhibited to >95% at 60°C when adding 200 ng UPstart *Taq* Antibody (1 U) to one unit *Taq* DNA polymerase.

Mouse genomic DNA contamination assay

An UPstart *Taq* Antibody sample is analyzed for the presence of contaminating mouse genomic DNA. No mouse genomic 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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