

UPstart™ Plus *Taq* Antibody, 1 mg/ml

DESCRIPTION

biotechrabbit™ UPstart Plus *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 Plus *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 Plus *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
<i>Taq</i> DNA Polymerase, 5 U/μl (i.e. BR0100101)	20 μl	100 U
UPstart Plus <i>Taq</i> Antibody	20 μl	100 U

*Gives 100 reactions with 1 unit Hot-start *Taq* DNA polymerase per reaction.*

For one reaction use:

<i>Taq</i> DNA Polymerase / UPstart Plus <i>Taq</i> Antibody pre-mix	0.4 μl	1 U Hot-Start <i>Taq</i> 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 MgCl₂ solution and dNTPs.
- Keep all reagents and reactions on ice.
- When setting up multiple reactions, prepare a master mix of water, buffer, dNTPs, UPstart Plus *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.

UPstart™ Plus Taq Antibody, 1 mg/ml

COMPONENT	VOLUME	FINAL CONCENTRATION
Taq DNA Polymerase, 5 U/μl	0.2 μl	1 U
UPstart Plus Taq Antibody	0.2 μl	1 U
<i>Mix Taq DNA polymerase and UPstart Plus Taq Antibody and incubate at room temperature for 15 min. Alternatively use pre-mixed Taq DNA polymerase and UPstart Plus Taq Antibody.</i>		
10× Reaction Buffer	5 μl	1×
50 mM MgCl ₂	Variable (standard 1.5 μl)	1.5 mM
<i>Higher than 2 mM MgCl₂ might increase yield but reduce fidelity</i>		
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
<i>Use 0.01–1 ng for plasmid or phage DNA and 0.1–1 μg for genomic DNA</i>		
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 cyclers.

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
<i>Approximately 5°C below T_m of primers</i>			
Extension	72°C	30–60 s/kb	25–35
Final extension	72°C	5 min	1
<i>To extend all incomplete PCR products</i>			
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.

UPstart™ Plus Taq Antibody, 1 mg/ml

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 Plus Taq Antibody (200 ng of the antibody) will ensure inhibition of Taq DNA polymerase.

Quality Control

Functional Assay

UPstart Plus 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 Plus Taq Antibody (1 U) to one unit Taq DNA polymerase.

Mouse genomic DNA contamination assay

An UPstart Plus 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.

CONTACT BIOTECHRABBIT

biotechrabbit GmbH
Volmerstr. 9a
12489 Berlin, Germany

info@biotechrabbit.com
support@biotechrabbit.com
www.biotechrabbit.com

Phone: +49 30 555 7821-10
Fax: +49 30 555 7821-99



Legal Disclaimer and Product Use Limitation

Purchase of product does not include a license to perform any patented applications; therefore it is the sole responsibility of users to determine whether they may be required to engage a license agreement depending upon the particular application in which the product is used. This product was developed, manufactured, and sold for *in vitro* use only. It is not suitable for administration to humans or animals.

Trademarks: biotechrabbit™, UPstart™ (biotechrabbit GmbH).

valid from 27.11.2019