

# Titan HotTaq DNA Polymerase

Cat. No.	Pack Size	Conc.
BTS0002	50 U SAMPLE	5 U/μl
BT10201	500 U	5 U/µI
BA00202	1000 U	5 U/µI

## Storage & Shipping:

Store at -20°C, shipping at room temperature.

Shipping and temporary storage for up to 1 month at room temperature has no detrimental effects on the quality of Titan HotTaq DNA Polymerase.

## **Reagents Provided:**

- Titan HotTaq DNA Polymerase
- 10x Reaction Buffer B1 (Mg<sup>2+</sup>, detergent free): Tris-HCl and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>.
- 10x Reaction Buffer B2 (Mg<sup>2+</sup> free): Tris-HCl, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and detergent.
- 25 mM MgCl<sub>2</sub>
- 10x Enhancer

Additive that facilitates amplification of difficult templates (e.g. GC-rich DNA templates). Enhancer should be used at a defined working concentration (1x, 2x or 3x solution).

Enhancer is NOT a reaction buffer and should be used ONLY IF non-specific amplifications occur.

# **Description:**

Titan HotTaq DNA Polymerase is a chemically modified Titan Taq DNA Polymerase. At ambient temperatures it is inactive, having no polymerization activity. Titan HotTaq DNA Polymerase is activated by a 15 min incubation step at 95°C. This prevents extentension of non-specifically annealed primers and primer-dimers formed at low temperatures during PCR setup. The enzyme has  $5'\rightarrow 3'$  polymerization-dependent exonuclease replacement activity but lacks  $3'\rightarrow 5'$  exonuclease activity.

#### Source:

Purified from an *E.coli* strain that carries an overproducing plasmid containing a modified gene of *Thermus aquaticus* DNA Polymerase.

## Storage and Dilution buffer:

50% glycerol (v/v), 20 mM Tris-HCl pH 8.7 at 25°C, 100 mM KCl, 0.1 mM EDTA and stabilizers.

# **Quality data:**

The enzyme is free of nicking and priming activities, exonucleases and non-specific endonucleases. SDS/PAGE - 95 kD band, >98% pure. Activity and stability tested via thermo cycling. The error rate per nucleotide per cycle is  $\sim 2.5 \times 10^{-5}$ ; the accuracy is  $\sim 4 \times 10^{4}$ . Estimated half-life at 95°C is 1.5 hours.

## **Unit definition:**

One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nmol of dNTPs into an acid-insoluble form in 30 minutes at 74°C.



## Recommended PCR reaction mix:

Component	Volume	Final conc.
Titan HotTaq (5 U/μl)	0.4-1.0 μΙ	0.02-0.05 U/µl (2-5 U)
10x Buffer B1 or B2	10 μΙ	1x
25 mM MgCl <sub>2</sub>	6-10 µl	1.5-2.5 mM
20 mM dNTP mix	1 μΙ	200 μΜ
Primer Forward (10 pmol/μl)	1-3 µl	0.1-0.3 μM
Primer Reverse (10 pmol/µI)	1-3 µl	0.1-0.3 μM
DNA template	5-20 µl	5-100 ng/µl
10x Enhancer  Not for standard PCR	0, 10, 20 or 30 μl	1x, 2x or 3x
H <sub>2</sub> O PCR grade	Up to 100 μl	
Total	100 μΙ	

# **Recommended PCR cycles:**

Cycle step	Temp.	Time	Cycles
Initial denaturation	95°C	12-15 min	1
Denaturation	95°C	30-60 s	
Annealing	50-68°C	30-60 s	26-35
Elongation	72°C	1-4 min	
Final elongation	72°C	5-10 min	1

**IMPORTANT:** To activate the polymerase, include an incubation step **at 95°C for 12 - 15 minutes** at the beginning of the PCR cycle. Annealing temperature should be 2-6°C lower than the primer melting temperature. Elongation time should be ~1 min/1 kb.

# Safety warnings and precautions:

This product is designed for research purposes and *in vitro* use only. According to common laboratory safety practice, it is recommended to wear protective clothing, gloves and safety glasses. Please refer to www.bioatlas.com for Material Safety Data Sheet of the product.

Some applications this product is used in may require a license which is not provided by the purchase of this product. Users should obtain the license if required.