A&A BIOTECHNOLOGY
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Manual

T4 DNA polymerase

Concentration 3 U/µl

catalog #	size
1031-100	100 U
1031-500	500 U

For research use only.

Guarantee
A&A Biotechnology provides guarantee on this product.
The company does not guarantee correct performance of this kit in the event of:

- not adhering to the supplied protocol
- use of not recommended equipment or materials
- use of other reagents than recommended or which are not a component of the product
- use of expired or improperly stored product or its components

Specification

form	solution
concentration	3 U/µl
unit definition	One unit of the enzyme catalyzes the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction in 30 min at 37 °C.
applications	• Blunting of dsDNA ends by filling 5'-overhangs or removal of 3'-overhangs. • Blunting of PCR products containing 3'-dA overhangs (after amplification with Taq DNA polymerase) • Synthesis and labeling of DNA probes by the replacement reaction. • Oligonucleotide-directed site-specific mutagenesis. • Ligation-independent cloning of PCR products.
source	recombinant
storage buffer	100 mM KPO_4, 1 mM DTT, 50% glycerol, pH 6.5 @ 25°C

Description

T4 DNA Polymerase is a template-dependent polymerase that catalyzes 5'-3' synthesis from primed single-stranded DNA. The enzyme also has 3'-5 'exonuclease activity. The T4 DNA polymerase has no 5'-3 'exonuclease activity.

The 3'-5 'exonuclease activity is much higher for ssDNA compared to dsDNA.

T4 DNA polymerase is active in most buffers used in PCR, reverse transcription, ligation buffer and in most buffers for restriction enzymes

Contents

	1031-100	**1031-500**	**storage**
T4 DNA polymerase	35 µl	5 x 35 µl	-20 °C
T4 reaction buffer	220 µl	1 x 1.1 ml	-20 °C
10x reaction buffer: 500 mM NaCl, 100 mM Tris-HCl, 100 mM $MgCl_2$, 1 mg/ml BSA, pH 8.0			

Additional equipment and reagents

- dNTPs
- EDTA (optional)
- sterile water

Protocol for blunting of 5'- or 3'-overhangs

1. Prepare the following reaction mixture:

component	amount
T4 reaction buffer	2 µl
linear DNA or PCR product	1 µg
dNTPs mix, 2 mM each	1 µl (100 µM final concentration)
T4 DNA polymerase	0.3 µl (1 U)
sterile water, nuclease free	to 20 µl

Note: Use 1 U of T4 DNA Polymerase per microgram DNA or PCR product.

2. Thoroughly mix, briefly centrifuge and incubate for **15 min** at **12 °C** or for **5 min** at **room temp.**

Note: Do not exceed the incubation time.

3. Stop reaction by adding **0.6 µl** of **EDTA** (0.5 M) or heating to **75 °C** for **20 min.**

4. Briefly vortex and centrifuge for 3-5 s.

5. Store the prepared DNA or PCR product in the reaction mixture at -20 °C if the next step cannot be performed immediately.

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