Invitrogen
life technologies

T4 DNA Polymerase

Cat. No.	Size:
18005-017	50 units
18005-025	250 units

Conc. 5 U/µl **Store at -20°C** in a non-frost-free freezer

Description

T4 DNA Polymerase is purified from a T4 N82-infected E. coli by a modification of Challberg and Englund. This enzyme exhibits 5′→3′ DNA polymerase and 3′→5′ exonuclease activity (1,2,3). It may be used to fill restriction endonuclease termini and in replacement synthesis for the generation of radioactive probes (1,2).

Amount

Component	50-U kit	250-U kit
T4 DNA Polymerase	10 µl	50 µl
5X T4 DNA Polymerase Buffer	1 ml	1 ml

Unit Definition

One unit incorporates 10 nmol of deoxyribonucleotide into acid-precipitable material in 30 minutes at 37°C.

Storage Buffer

100 mM potassium phosphate (pH 6.5), 10 mM 2-mercaptoethanol, 50% (v/v) glycerol

5X T4 DNA Polymerase Buffer

165 mM Tris-acetate (pH 7.9), 330 mM sodium acetate, 50 mM magnesium acetate, 5 mM DTT.

Store buffer at -20°C.

Part no. 18005.pps Rev. date: 07/10/03

For technical questions about this product, call the Invitrogen Tech-Line℠ U.S.A. 800 955 6288

Quality Control

This product has passed the following quality control assays: absence of detectable endodeoxyribonuclease and phosphatase activities; performance in a replacement synthesis reaction.

The enclosed buffers were assayed with the enzyme and met quality control specifications.

Protocol for Generating Blunt Ends

The following reaction conditions can be used to generate blunt ends using 0.5–2.5 µg of double-stranded, linear DNA.

1. To an autoclaved, 1.5-ml microcentrifuge tube on ice, add:

Component	Amount
5X T4 DNA polymerase buffer	20 µl
0.5 mM dNTP mix	20 µl
DNA	0.5–2.5 µg
T4 DNA polymerase (5 U/µl)	2 µl
Autoclaved, distilled water	to 100 µl

2. Mix gently, and incubate at 11°C for 15 min.
3. Place reaction on ice.
4. Terminate reaction by phenol extraction, chloroform extraction, and ethanol precipitation. Heat inactivation is not recommended, as heat inactivation may cause ends to "breathe" and be more susceptible to 3′ to 5′ exonuclease activity.

References

1. O'Farrell, P. (1981) *Focus 3*. 1
2. Deen, K.C., Landers, T. A., and Berninger, M. (1983) *Anal. Biochem. 135*, 456.
3. Challberg, M.D. and Englund, P.T. (1979) *J. Biol. Chem. 254*, 7820.