Thermo SCIENTIFIC

PRODUCT INFORMATION

T7 DNA Polymerase

Pub. No. MAN0012017
Rev. Date 13 June 2016 (B.00)

Lot: _ **Expiry Date: _**

Components	#EP0081 300 U
Concentration	10 U/µL
10X Reaction Buffer	0.4 mL

Store at -20 °C

www.thermofisher.com

For Research Use Only. Not for use in diagnostic procedures.

Description

T7 DNA Polymerase, a template depended DNA polymerase, catalyzes DNA synthesis in the 5'→3' direction. It is a highly processive DNA polymerase allowing continuous synthesis of long stretches of DNA. The enzyme also exhibits a high 3'→5' exonuclease activity towards single- and double-stranded DNA.

Applications

- Purification of covalently closed circular DNA by removal of residual genomic DNA.
- Primer extension reactions on long templates (1).
- DNA 3'-end labeling (1).
- Strand extensions in site-directed mutagenesis (2).
- Fill-in blunting of 5'-overhang DNA.
- Second strand synthesis of cDNA (3).
- *In situ* detection of DNA fragmentation associated with apoptosis (4).

Source

Two *E.coli* strains, one with the cloned gene 5 of bacteriophage T7, and the other – *trxA* gene of *E.coli.*

Molecular Weight

T7 DNA Polymerase is composed of two subunits: 84.0 kDa polypeptide specified by gene 5 of bacteriophage T7, and 12.0 kDa thioredoxin specified by *trxA* gene of *E.coli.*

Definition of Activity Unit

One unit of the enzyme catalyzes the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction in 30 min at 37 °C.

Storage Buffer

The enzyme is supplied in: 20 mM potassium phosphate (pH 7.4), 1 mM DTT, 0.1 mM EDTA and 50% (v/v) glycerol.

10X Reaction Buffer

400 mM Tris-HCl (pH 7.5 at 25 °C), 100 mM $MgCl_2$, 10 mM DTT.

Inhibition and Inactivation

- Inhibitors: metal chelators, modification reagents (acetic anhydride, N-ethylmaleimide inactivate the 3'→5' exonuclease activity but not the polymerase activity) (5).
- Inactivated by heating at 75 °C for 10 min.

Note

- Assays at 37 °C require only short incubation times (6).
- Activity in Thermo Scientific REase Buffers, % (in comparison to activity in assay buffer)

Buffers	Activity, %
for restriction enzymes:	
Thermo Scientific™ FastDigest™, FastDigest Green, G, O, R, 1X Thermo Scientific™ Tango™, 2X Tango, EcoRI, KpnI	100
B, BamHI, Ecl136II, PacI, SacI	75-100

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

No detectable degradation was observed after incubation of supercoiled plasmid DNA with T7 DNA polymerase.

Quality authorized by: Jurgita Zilinskiene

(continued on back page)

References

1. Sambrook, J., Russell, D.W., Molecular Cloning: A Laboratory Manual, Third edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001.
2. Bebenek, K., Kunkel, T.A., The use of native T7 DNA polymerase for site-directed mutagenesis, Nucleic Acids Res., 17, 5408, 1989.
3. Bodescot, M., Brison, O., Efficient second-strand cDNA synthesis using T7 DNA polymerase, DNA and Cell Biology, 13, 977-985, 1994.
4. Wood, K.A., et al., *In situ* labeling of granule cells for apoptosis-associated DNA fragmentation reveals different mechanisms of cell loss in developing cerebellum Neuron, 11, 621-632, 1993.
5. Eun, H-M., Enzymology Primer for Recombinant DNA Technology, Academic Press, Inc., 1996.
6. Bodescot, M., Brison, O., T7 DNA polymerase requires unusual reaction conditions for blunt-ending activity, Anal. Biochemistry, 216, 234-235, 1994.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively *for research purposes and in vitro use only.* The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
Please refer to www.thermofisher.com for Material Safety Data Sheet of the product.