

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/ μ L
10X Reaction Buffer	0.4 mL

Store at -20 °C

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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 MgCl₂, 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, PaeI, 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

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References

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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.

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