

PRODUCT INFORMATION

T4 DNA Polymerase

#EP0062 **500 U**

Lot: _ **Expiry Date: _**

Concentration: 5 U/ μ L

Supplied with: 2 x 1 mL of 5X Reaction Buffer

Store at -20°C

In total 3 vials.

Description

T4 DNA Polymerase, a template-dependent DNA polymerase, catalyzes 5'→3' synthesis from primed single-stranded DNA. The enzyme has a 3'→5' exonuclease activity, but lacks 5'→3' exonuclease activity.

Applications

- Blunting of DNA ends: fill-in 5'-overhangs or/and removal of 3'-overhangs (1, 2), see protocol on back page.
- Blunting of PCR products with 3'-dA overhangs (6).
- Synthesis of labeled DNA probes by the replacement reaction (3).
- Oligonucleotide-directed site-specific mutagenesis (4).
- Ligation-independent cloning of PCR products (5).

Source

E.coli cells with a cloned gene 43 of bacteriophage T4.

Molecular Weight

104 kDa monomer.

Definition of Activity Unit

One unit of the enzyme catalyzes the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction (adsorbed on DE-81) in 30 min at 37°C.

Enzyme activity is assayed in the following mixture: 67 mM Tris-HCl (pH 8.8), 6.7 mM MgCl₂, 1 mM DTT, 16.7 mM (NH₄)₂SO₄, 0.2 mg/mL BSA, 0.033 mM of each dNTP, 0.4 MBq/mL [³H]-dTTP and 0.2 mM heat-denatured and nuclease-digested calf thymus DNA.

Storage Buffer

The enzyme is supplied in: 20 mM potassium phosphate (pH 7.5), 200 mM KCl, 2 mM DTT, and 50% (v/v) glycerol.

5X Reaction Buffer

335 mM Tris-HCl (pH 8.8 at 25°C), 33 mM MgCl₂, 5 mM DTT, 84 mM (NH₄)₂SO₄.

Inhibition and Inactivation

- Inhibitors: metal chelators, nucleotide analogs 2(*p-n*-butylanilino)-dATP, N²-(*p-n*-butylphenyl)-dGTP), SH-blocking compounds (7).
- Inactivated by heating at 75°C for 10 min.

Note

- The 3'→5' exonuclease activity of T4 DNA Polymerase is stronger on single-stranded DNA than on double-stranded DNA and greater (more than 200 times) than that of DNA Polymerase I, *E.coli* (1).
- Activity in Thermo Scientific buffers, % (in comparison to activity in assay buffer)

| Buffers | Activity, % |
|--|---------------|
| for restriction enzymes: Thermo Scientific FastDigest, FastDigest [®] Green, O, R, 1X Thermo Scientific Tango, 2X Tango [™] , BamHI, EcoRI, Ecl136II, KpnI, PaeI, SacI B, G | 100 75-100 |
| for PCR buffers: <i>Taq</i> buffer with KCl and <i>Pfu</i> buffer <i>Taq</i> buffer with (NH ₄) ₂ SO ₄ | 50 100 |
| RT buffers | 100 |

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

No conversion of covalently closed circular DNA to nicked DNA was detected after incubation of 10 units of T4 DNA Polymerase with 1 µg of pUC19 DNA for 4 hours at 37°C.

Quality authorized by:

 Jurgita Zilinskiene

(continued on back page)

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

1. Prepare the following reaction mixture:

| | |
|--------------------------------------|--|
| 5X reaction buffer | 4 μ L |
| Linear DNA or PCR product | 1 μ g |
| dNTP Mix, 2 mM each (#R0241) | 1 μ L (0.1 mM final concentration) |
| T4 DNA Polymerase | 0.2 μ L (1 U) |
| Water, nuclease-free (#R0581) | to 20 μ L |

2. Mix thoroughly, spin briefly and incubate at 11°C for 20 min or at room temperature for 5 min.

3. Stop the reaction by heating at 75°C for 10 min.

References

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3. Challberg, M.D., Englund, P.T., Specific labeling of 3'-termini with T4 DNA polymerase, Methods Enzymol., 65, 39-43, 1980.
4. Kunkel, I.A., et al., Rapid and efficient site-specific mutagenesis without phenotypic selection, Methods Enzymol., 154, 367-382, 1987.
5. Haun, R.S., et al., Rapid, reliable ligation-independent cloning of PCR products using modified plasmid vectors, BioTechniques, 13, 515-518, 1992.
6. Wang, K., et al., A simple method using T4 DNA polymerase to clone polymerase chain reaction products, BioTechniques, 17, 236-238, 1994.
7. Eun, H-M., Enzymology Primer for Recombinant DNA Technology, Academic Press, Inc., 1996

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.thermoscientific.com/onebio for Material Safety Data Sheet of the product.

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