Thermo SCIENTIFIC

PRODUCT INFORMATION

T4 DNA Polymerase

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Lot: _ **Expiry Date: _**

Components	#EP0061 100 U	#EP0062 500 U
Concentration	5 U/µL	5 U/µL
5X Reaction Buffer	0.35 mL	2 × 1 mL

Store at -20 °C

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Description

T4 DNA Polymerase, a template-depended 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 in 30 min at 37 °C.

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 $MgCl_2$, 5 mM DTT, 84 mM $(NH_4)_2SO_4$.

Inhibition and Inactivation

- Inhibitors: metal chelators, nucleotide analogs 2(*p-n*-butylanilino)-dATP, N^2-(*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, EcI136II, KpnI, PacI,	100
SacI	75-100
B, G	
for PCR buffers:	
Taq buffer with KCl and *Pfu* buffer	50
Taq buffer with $(NH_4)_2SO_4$	100
RT buffers	100

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

No detectable degradation was observed after incubation of supercoiled plasmid DNA with T4 DNA Polymerase.

Quality authorized by: Jurgita Zilinskiene

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

1. Prepare the following reaction mixture:

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

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

1. Sambrook, J., Russell D.W., Molecular Cloning: A Laboratory Manual, the third edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001.
2. Ausubel, F.M., et al., Current Protocols in Molecular Biology, vol. 1, John Wiley & Sons, Inc., Brooklyn, New York, 3.5.11-3.5.12, 1994-2004.
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

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