

CERTIFICATE OF ANALYSIS

T4 DNA Polymerase

#EP0062 500u

Lot: **Quality guaranteed:**

Concentration: 5u/μl

Supplied with: 2x1.0ml of 5X Reaction Buffer

Store at -20°C

2

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.

Source

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

Unit Definition

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

Activity Assay

67mM Tris-HCl (pH 8.8), 6.7mM MgCl₂, 1mM DTT, 16.7mM (NH₄)₂SO₄, 0.2mg/ml BSA, 0.033mM of each dNTP, 0.4MBq/ml [³H]-dTTP and 0.2mM heat-denatured and nuclease-digested calf thymus DNA.

Storage Buffer

20mM potassium phosphate (pH 7.5), 200mM KCl, 2mM DTT, and 50% glycerol.

5X Reaction Buffer

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

Applications

- Blunting DNA with 5'- or 3'-protruding termini (1, 2), see protocol on back page.
- Synthesis of labeled DNA probes by the replacement reaction (3).
- Synthesis of the second strand in site-directed mutagenesis (4).
- Cloning of PCR products (5, 6).

Inactivation

By heating at 70°C for 10min.

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 Fermentas REase Buffers, % (in comparison to activity in assay buffer)

B	G	O	R	Tango™		BamHI	Ecl136II, Sacl	EcoRI	KpnI
				1X	2X				
75-100	75-100	100	100	100	100	100	100	100	100

QUALITY CONTROL ASSAY DATA

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 10 units of T4 DNA Polymerase with 1µg of pBR322 DNA in 50µl of reaction buffer for 4 hours at 37°C.

Quality authorized by:

 Dalia Cesiuliene

DE-81 – Whatman anion exchange chromatography paper having diethylaminoethyl functional groups.

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(continued on back page)

Protocol for Blunting DNA with 5'- or 3'-protruding Termini

- ❶ Prepare the following reaction mixture:

5X reaction buffer	4 μ l
digested DNA	1 μ g
2mM dNTP Mix	1 μ l
	(0.1mM – final concentration)
T4 DNA Polymerase	1u
water, nuclease-free	to 20 μ l

- ❷ Incubate the mixture at 11°C for 20 minutes or at room temperature for 5 minutes.
- ❸ Stop the reaction by heating at 70°C for 10 minutes.

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.

Related Products

- 2mM dNTP Mix #R0241
#R0242
- 10mM dNTP Mix #R0191
#R0192
- dNTP Set #R0181
#R0182
#R0186
- Water, nuclease-free #R0581
#R0582

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.fermentas.com for Material Safety Data Sheet of the product.