T4 DNA Polymerase

Catalog #	3221	3222
Package Size	125 units	500 units
Volume	50 µl	100 µl
Concentration	5 units/µl	

Description

Intact Genomics T4 DNA Polymerase has both a DNA-dependent DNA polymerase activity and a potent 3′→5′ exonuclease activity.

Physical Purity

The purity of this enzyme is > 95% homogeneity as determined by SDS-PAGE using Coomassie® Blue staining (see figure below).

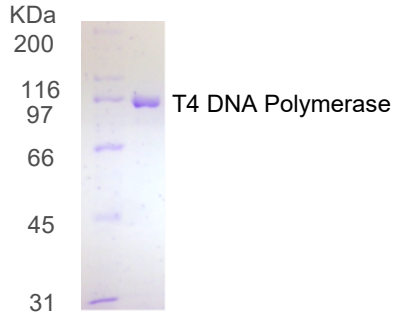

Figure: Lane 1. Protein Marker
Lane 2. T4 DNA Polymerase

Product Source

E.coli cells with a cloned gene of bacteriophage T4 DNA Polymerase.

Applications

- 3′-overhang removal to form blunt ends (1, 2).
- 5′-overhang fill-in to form blunt ends (1, 2).
- Probe labeling using replacement synthesis (2).
- DNA library preparation for Next-generation sequencing.
- Ligation-independent cloning of PCR products.
- Second strand synthesis in site-directed mutagenesis (3).

Product Includes

1) T4 DNA Polymerase
2) 10x T4 DNA Polymerase Buffer

Storage Temperature

–20 °C

Storage Buffer

50 mM Tris-HCl,
50 mM KCl,
1 mM DTT,
0.1 mM EDTA,
50% Glycerol, pH 7.5 @ 25 ºC

10X T4 DNA Polymerase Reaction Buffer

500 mM Tris-HCl
100 mM MgCl2
50 mM dithiothreitol
pH 7.5 @ 25°C.

Unit Definition

One unit of T4 DNA Polymerase converts 10 nmol of dNTPs into acid-insoluble material in 30 minutes at 37°C under standard assay conditions.

Inactivation

Inactivated by heating at 70°C for 15 min.

Quality Control Assays

T4 DNA Polymerase is free from detectable endonuclease and RNase activities.

Protocol

Blunting ends by 3’ overhang removal or 3’ recessed end fill-in:

1. Dissolve DNA in 1x reaction buffer supplemented with 100 µM dNTPs.
2. Add 1 unit T4 DNA Polymerase per µg DNA.
3. Incubate at room temperature for 5-30 minutes. Stop reaction by heating at 70°C for 20 minutes.

References

Tabor, S. and Struhl, K. (1989). DNA-Dependent DNA Polymerases. Current Protocols in Molecular Biology. 3.5.10-3.5.12. New York: John Wiley & Sons, Inc.
Sambrook, J. et al. (1989). Molecular Cloning: A Laboratory Manual. (2nd ed.), 5.44-5.47. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
Kunkel, T.A. et al. (1987). Methods Enzymol. 154, 367-382.