T4 DNA Polymerase

Intact Genomics®

Catalog #	3222
Package Size	500 units
Volume	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).

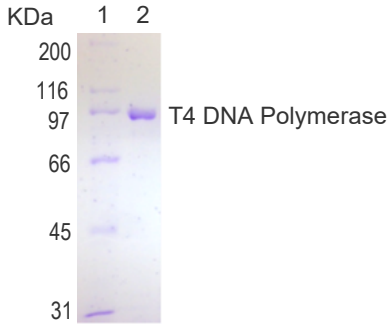

Figure: Lane 1. Protein Marker
Lane 2. T4 DNA Polymerase

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 (pH 7.5), 0.1 mM EDTA,
1 mM β-mercaptoethanol, 1 mM DTT , 25% (v/v) glycerol.

10X T4 DNA Polymerase Reaction Buffer

500 mM Tris-HCl, 100 mM $MgCl_2$, 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.

Related Products

- Taq DNA Polymerase (Cat.# 3243)
- Taq DNA Polymerase 2x Premix (Cat.# 3249)
- T4 DNA Ligase (Cat.# 3212)

Technical Support

Intact Genomics is committed to supporting the worldwide scientific research community by supplying the highest quality reagents. Each new lot of our products is tested to ensure they meet the quality standards and specifications designated for the product. Please follow the instructions carefully and contact us if additional assistance is needed. We appreciate your business and your feedback regarding the performance of our products in your applications.