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Not for use in diagnostic procedures.



T4 DNA Polymerase **from T4 plasmid pTL43W infected** ***Escherichia coli* 71-18**

 **Version: 19**

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Deoxynucleoside triphosphate: DNA deoxynucleotidyltransferase (DNA-directed)

Cat. No. 11 004 786 001 100 U
 1 U/μl

Store the product at –15 to –25°C.

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1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Catalog Number	Content
1	T4 DNA Polymerase	▪ 1 U/μl	11 004 786 001	1 vial, 100 U
		▪ Enzyme storage buffer: 10 mM Tris-HCl, 100 mM NaCl, 2.5 mM MgCl ₂ , 0.5 mM EDTA, 2 mM dithioerythritol, 50% glycerol (v/v), pH 8.0 (+4°C).	11 004 794 001	1 vial, 500 U
2	Incubation Buffer, 5x conc. for T4 DNA Polymerase	Buffer composition: 250 mM Tris-HCl, 75 mM (NH ₄) ₂ SO ₄ ,	11 004 786 001	1 vial, 1 ml
		35 mM MgCl ₂ , 0.5 mM EDTA, 50 mM 2-mercaptoethanol, 0.1 mg/ml bovine serum albumin (BSA), pH 8.8 (+25°C).	11 004 794 001	1 vial, 1 ml

1.2. Storage and Stability

Storage Conditions (Product)

When stored at –15 to –25°C, the product is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	T4 DNA Polymerase	Store at –15 to –25°C.
2	Incubation Buffer, 5x conc.	

1.3. Additional Equipment and Reagent required

For polymerization reactions

- Set of Deoxynucleotides* or
- dATP*
- dCTP*
- dGTP*
- dTTP*

For gap-filling in site-directed mutagenesis

- T4 Gene 32 Protein*

1.4. Application

T4 DNA Polymerase is used in a variety of techniques:

- Labels 3' termini of DNA. Extensive labeling is achieved by the replacement reaction in which the 3'-exonuclease activity of the enzyme first digests dsDNA to produce molecules with recessed 3' termini. On the subsequent addition of labeled dNTPs, the polymerase activity of T4 DNA Polymerase extends the 3' ends along the length of the template. Exonuclease III from *E. coli* can be used to create partially single-stranded dsDNA for subsequent polymerization reactions.
- Molecules labeled to high specific activity are used chiefly as hybridization probes. They have two advantages over probes prepared by nick translation: they lack artificial hairpin structures and can easily be converted into strand-specific probes by cleavage with suitable restriction endonucleases.
- In combination with T4 gene 32 protein*, T4 DNA Polymerase is used for gap-filling in site-directed mutagenesis experiments.
- Fill-in reactions.

Product Description

T4 DNA Polymerase is a DNA-dependent DNA polymerase that catalyzes the polymerization of deoxynucleoside-5'-triphosphates to the hydroxyl termini of recessive ends.

i *Blunt ended DNA cannot serve as template for the polymerization reaction.*

- The polymerization of T4 DNA Polymerase requires DNA with 5'-protruding ends and a high concentration of dNTPs. The enzyme carries an extremely active 3'→5' exonuclease that shows a strong specificity for ssDNA and lacks a 5'→3'-exonuclease activity. Therefore, nicked duplex DNA cannot serve simultaneously as template and primer for polymerization.
- The addition of T4 Gene 32 Protein* facilitates strand displacement and allows T4 DNA Polymerase to replicate the nicked duplex.

⚠ *Do not use low levels of dNTPs for polymerization reactions. Once the dNTPs are exhausted, the exonuclease activity will degrade the DNA.*

2. How to Use this Product

2.1. Before you Begin

Mg²⁺ Concentration

Optimal Mg²⁺ approximately 6 mM.

Working Solution

Incubation buffer

For polymerization reactions, supplement the Incubation Buffer with 33 µmol/l each dATP*, dCTP*, dGTP* and dTTP* (final concentration).

i This buffer is optimal for the polymerase and exonuclease activities of T4 DNA Polymerase.

2.2. Parameters

Molecular Weight

114,000 Da, single polypeptide

pH Optimum

8 to 9 (at pH 7.5 and 9.7, approximately 50% activity is found)

Purity

≥10 µg of T4 DNA polymerase migrate as a single band in SDS-polyacrylamide gel electrophoresis.

Specific Activity

≥5 U/µg

Volume Activity

≥1 U/µl of T4 DNA Polymerase is the amount of enzyme activity that incorporates 10 nmol dNTP into acid-precipitable DNA products in 30 minutes at +37°C.

3. Additional Information on this Product









3.1. Quality Control

For lot-specific certificates of analysis, see section, **Contact and Support**.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
 <i>Information Note: Additional information about the current topic or procedure.</i>	
 Important Note: Information critical to the success of the current procedure or use of the product.	
   etc.	Stages in a process that usually occur in the order listed.
   etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.

Editorial changes.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Deoxynucleoside Triphosphate Set	4 x 250 µl, 4 x 25 µmol, 100 mM	11 969 064 001
	4 x 1,250 µl, 4 x 125 µmol, 100 mM	03 622 614 001
dATP	250 µl, 25 µmol, 100 mM 6,250 standard PCR assays of 20 µl each.	11 934 511 001
	1,250 µl, 125 µmol, 100 mM 31,250 standard PCR assays of 20 µl each.	11 969 013 001
dGTP	250 µl, 25 µmol, 100 mM 6,250 standard PCR assays of 20 µl each.	11 934 538 001
	1,250 µl, 125 µmol, 100 mM 31,250 standard PCR assays of 20 µl each.	11 969 030 001
	4 x 1,250 µl, 4 x 125 µmol, 100 mM 125,000 standard PCR assays of 20 µl each.	03 732 703 001
dCTP	250 µl, 25 µmol, 100 mM 6,250 standard PCR assays of 20 µl each.	11 934 520 001
	1,250 µl, 125 µmol, 100 mM 31,250 standard PCR assays of 20 µl each.	11 969 021 001
	4 x 1,250 µl, 4 x 125 µmol, 100 mM 125,000 standard PCR assays of 20 µl each.	03 732 690 001
dTTP	250 µl, 25 µmol, 100 mM 6,250 standard PCR assays of 20 µl each.	11 934 546 001
	1,250 µl, 125 µmol, 100 mM 31,250 standard PCR assays of 20 µl each.	11 969 048 001
T4 Gene 32 Protein	100 µg	10 972 983 001
	500 µg	10 972 991 001

4.4. Trademarks

All product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:

List of biochemical reagent products and select the corresponding product catalog.

4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed

