T4 DNA Polymerase

Cat. No.: FP1508928 | Pack size: 300 U / 3 KU / 30 KU | Storage: -20 °C; avoid repeated freeze/thaw

Overview

T4 DNA Polymerase is purified from E. coli carrying a highly expressed T4 DNA Polymerase gene.

This template-dependent DNA polymerase catalyzes DNA synthesis in the 5'->3' direction on primer-bound single-stranded DNA templates. It has strong 3'->5' exonuclease activity, no 5'->3' exonuclease activity, and is commonly used for DNA end blunting, second-strand synthesis in site-directed mutagenesis, and ligation-independent cloning of PCR products.

Key Features

- Template-dependent 5'->3' DNA polymerase activity
- Strong 3'->5' exonuclease activity for trimming protruding 3' ends
- No 5'->3' exonuclease activity
- Suitable for gap filling and conversion of 5' or 3' overhangs to blunt ends
- Useful for probe labeling, subcloning after single-strand deletion, and second-strand synthesis in site-directed mutagenesis

Contents & Storage

Cat. No.	Component	300 U	3 KU	30 KU	Storage
FP1508928A	T4 DNA Polymerase (3 U/µL)	100 µL	1.0 mL	10 mL	-20 °C
FP1508928B	10x T4 DNA Polymerase Buffer	1.0 mL	10x1.0 mL	100 mL	-20 °C
FP1508928C	Solution I (100x)	100 µL	1.0 mL	10 mL	-20 °C

Materials Required But Not Supplied

Item	Recommended Specification	Purpose
DNA substrate	Purified DNA with overhangs or gaps	End repair or blunting reaction
dNTP Mix	25 mM, user-supplied	DNA synthesis substrate
Nuclease-free water	DNase/RNase-free	Reaction-volume adjustment

Item	Recommended Specification	Purpose
Heat block or thermal cycler	12-37 °C and 75 °C capability	Reaction and inactivation
Ice bath or ice box	Clean molecular biology workflow	Reaction setup

Preparation Before Use

1. Thaw required reagents at 4 °C and keep them on ice during reaction setup.
2. Aliquot reagents when appropriate to avoid repeated freeze/thaw cycles.
3. Prepare the reaction mixture on ice and mix gently before incubation.

Protocol

Reaction System (End Repair Example)

Component	Volume	Final Concentration
10x T4 DNA Polymerase Buffer	5 µL	1x
dNTP Mix (25 mM)	0.4 µL	0.2 mM
Solution I (100x)	0.5 µL	1x
T4 DNA Polymerase (3 U/µL)	As required*	—
Reactants	As required*	—
ddH_2O	To 50 µL	—
Final Volume	50 µL	—

*10x T4 DNA Polymerase Buffer does not contain dNTP Mix; prepare dNTP Mix separately.

*As a general starting point, add 1 U of T4 DNA Polymerase per microgram of DNA substrate.

Reaction Procedure (End Repair Example)

Step Name	Temperature	Time
Reaction	12-37 °C	15-30 min
Inactivation	75 °C	20 min
Storage	4 °C	∞

Reaction temperature and time may be adjusted according to substrate amount and enzyme amount.

Storage & Handling

Store at -20 °C for long-term use. Upon receipt, aliquot if necessary and avoid repeated freeze/thaw cycles.

During use, keep enzyme on ice and return it to -20 °C immediately after setup.

Safety & Precautions

1. Because this enzyme has strong 3'->5' exonuclease activity, high reaction temperature, excessive enzyme, absence of dNTPs, or prolonged reaction time may excise bases from DNA ends and create recessed ends.
2. Wear lab coat and disposable gloves during operation.

Quality Control

QC Item	Method	Acceptable Range
Component completeness	Visual inspection on receipt	All listed components present; no leakage or visible damage
Enzyme performance	Functional amplification or end-repair assay	Meets product specification when used under recommended conditions
Storage integrity	Cold-chain and label check	Stored at the specified temperature and protected from repeated freeze/thaw cycles

Troubleshooting

Issue	Possible Causes	Corrective Action
Recessed DNA ends	Excessive enzyme, high temperature, no dNTPs, or prolonged incubation	Reduce enzyme amount or incubation time; include dNTPs when filling is required
Incomplete blunting	Insufficient enzyme or short reaction time	Increase enzyme within validated range or extend reaction time

Issue	Possible Causes	Corrective Action
Low downstream cloning efficiency	Residual enzyme or buffer incompatibility	Heat-inactivate, purify DNA if necessary, and confirm downstream buffer compatibility

Recommended Applications

Gap filling · 5' overhang filling · 3' overhang trimming · probe labeling · subcloning after single-strand deletion · second-strand synthesis in site-directed mutagenesis

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NORTH AMERICAN SALES, SUPPORT & GENERAL INQUIRIES	EU SALES, LOGISTICS & LOCALIZED SUPPORT
Aladdin Scientific Corporation	**Aladdin Biochem Deutschland GmbH**
14078 Meridian Parkway, Riverside, CA 92518, USA	Westring 2, 33142 Büren, Germany
Phone: 1-833-552-7181	**Phone:** +02951 9383958
Sales: sales@aladdinsci.com	**Support:** support.eu@aladdinsci.com
Customer Service: custserv@aladdinsci.com	

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