Certificate of Analysis

T4 DNA Polymerase:

Part No.	Size (units)
M421A	100
M421F	500

Enzyme Storage Buffer: 200mM potassium phosphate (pH 6.5), 2mM DTT and 50% glycerol.

T4 DNA Polymerase 10X Buffer: The 10X Buffer supplied with this enzyme has a composition of 250mM Tris-Acetate (pH 7.7), 1M potassium acetate, 100mM magnesium acetate and 10mM DTT.

Activity of T4 DNA Polymerase in Promega's Restriction Enzyme Buffers: The activity of T4 DNA Polymerase in restriction enzyme buffers B, C, E and MULTI-CORE™ Buffer is at least 70% of the value obtained under the conditions of the unit activity assay. Activity in other restriction buffers may be 50% or less of the value obtained under the conditions of the unit activity assay.

Heat-Inactivation: T4 DNA Polymerase may be inactivated by incubation at 75°C for 10 minutes.

Inhibitors: T4 DNA Polymerase is reported to be inhibited by –SH blocking agents (1).

Molecular Weight: 112kDa.

Source: Purified from an *E. coli* strain expressing a recombinant clone.

Storage Temperature: Store at –20°C. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. See the expiration date on the Product Information Label.

Unit Definition: One unit is defined as the amount of enzyme required to catalyze the incorporation of 55nmol of dTTP into acid-precipitable material in 30 minutes at 39°C using poly(dA):oligo(dT) as a substrate. See the unit concentration on the Product Information Label.

Quality Control Assays

Contaminant Activity

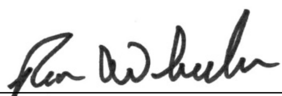
Endonuclease Assay: To test for endonuclease activity, 1µg of Type I supercoiled plasmid DNA is incubated with 40 units of T4 DNA Polymerase for one hour at 37°C in 1X restriction enzyme Buffer D. Following incubation, the supercoiled DNA is visualized on an ethidium bromide-stained agarose gel to verify the absence of visible nicking or cutting.

Single-Strand Endonuclease Assay: There must be no endonuclease activity detected when 1µg of M13mp18 DNA is incubated with 40 units of T4 DNA Polymerase for 1 hour at 37°C.

Physical Purity: The purity is >95% as judged by SDS-polyacrylamide gels with Coomassie® blue staining.

Reference

1. Kornberg, A. (1992) *DNA Replication,* W.H. Freeman and Company, San Francisco, CA.

Signed by: 

R. Wheeler, Quality Assurance

Part# 9PIM421

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Usage Information

I. Description

T4 DNA Polymerase can be used to fill 5´ protruding ends with labeled or unlabeled dNTPs (1) or for the generation of blunt ends from DNA molecules with 3´ overhangs (2).

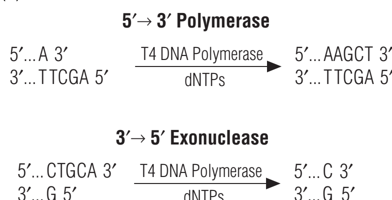

Figure 1. 5´→3´ polymerase, and 3´→5´ exonuclease activities of T4 DNA Polymerase.

II. Standard Applications

A. Filling 5´ Overhangs With Unlabeled dNTPs

1. Digest 0.5–2.0µg DNA (in a volume of 50µl) with a restriction enzyme that leaves a 5´ overhang.
2. Add 5 units of T4 DNA Polymerase/µg of DNA and 100µM of each dNTP. The recommended reaction buffer for T4 DNA Polymerase is 1X T4 DNA Polymerase Buffer. **Note:** T4 DNA Polymerase also functions well in many restriction enzyme reaction buffers.
3. Incubate at 37°C for 5 minutes. Stop the reaction by adding 2µl of 0.5M EDTA or by heating at 75°C for 10 minutes (3).

B. Filling 5´ Overhangs With Labeled dNTPs

1. After restriction enzyme digestion as described above, resuspend the DNA in restriction enzyme buffer **or** 1X T4 DNA Polymerase Buffer. Add 50µM of each unlabeled dNTP, 2µCi of the labeled [α-^{32}P]dNTP and 2.5 units of T4 DNA Polymerase in a total volume of 20µl.
2. Incubate for 5 minutes at 37°C. Stop the reaction by adding 1µl of 0.5M EDTA or by heating at 75°C for 10 minutes.

C. Converting a 3´ Overhang to a Blunt End

1. Digest 0.5–2.0µg of DNA (in a volume of 50µl) with a restriction enzyme that leaves a 3´ overhang. Resuspend the DNA in restriction enzyme buffer **or** 1X T4 DNA Polymerase Buffer. Add 5 units of T4 DNA Polymerase/µg of DNA and 100µM of each dNTP. Incubate at 37°C for 5 minutes.
2. Stop the reaction by adding 2µl of 0.5M EDTA or by heating at 75°C for 10 minutes. With high concentrations of dNTPs (100µM), degradation of the DNA will stop at duplex DNA; however, if the dNTP supply is exhausted, the very active exonuclease activity (200 times more active than that of DNA polymerase I) will degrade the double-stranded DNA (4).

D. Probe Synthesis

1. In a 20µl reaction volume, resuspend 0.5–2.0µg of linearized DNA in 1X T4 DNA Polymerase Buffer **or** restriction enzyme buffer. Add 1 unit of T4 DNA Polymerase for every microgram of DNA. The rate of exonuclease activity depends on the ratio of enzyme to DNA. After exonuclease digestion, add 1µl of a 2mM solution of each of the 3 unlabeled dNTPs, and add an amount of the [α-^{32}P]dNTP (400Ci/mmol) equivalent to the number of moles excised by the exonuclease.
2. Incubate the reaction at 37°C for 15 minutes. Stop the reaction by adding 1µl of 0.5M EDTA (5).

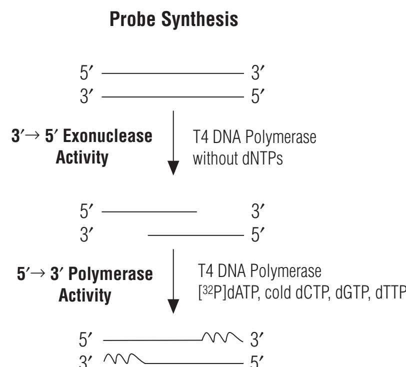

Figure 2. Probe synthesis with T4 DNA Polymerase.

III. References

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2. Burd, J.F. and Wells, R.D. (1974) Synthesis and characterization of the duplex block polymer d(C15A15)-d(T15G15). *J. Biol. Chem.* **249**, 7094–801.
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