

## Product Contents

### T4 DNA Polymerase<sup>(a)</sup>:

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.

Part# 9PIM421

Revised 5/04



**Promega**

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## 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.

<sup>(a)</sup>Licensed under U.S. Pat. No. 4,935,361.

<sup>(b)</sup>READIT® products may be covered by one or more of the following patents: U.S. Pat. Nos. 6,335,162, 6,159,693, 6,235,480, 6,312,902, 6,270,974, 6,277,578, 6,270,973, 6,268,146, 6,379,898, 6,391,551 and 6,653,078 and Australian Pat. Nos. 765428 and 766543. Other patents are pending.

Use of this method may or may not require performance of the polymerase chain reaction (PCR), which is covered by patents issued and applicable in certain countries. Accordingly, users of this product may be required to obtain a patent license from the appropriate patent owner.

<sup>(c)</sup>U.S. Pat. No. 5,552,302, European Pat. No. 0 422 217, Australian Pat. No. 646803 and Japanese Pat. Nos. 3009458 and 3366596 have been issued to Promega Corporation for the methods and compositions for production of human recombinant placental ribonuclease inhibitor. U.S. Pat. Nos. 4,966,964, 5,019,556 and 5,266,687, Australian Pat. Nos. 616881 and 641261 and other pending and issued patents, which claim vectors encoding a portion of human placental ribonuclease inhibitor, are exclusively licensed to Promega Corporation.

<sup>(d)</sup>U.S. Pat. No. 5,955,363 has been issued to Promega Corporation for a vector for in vitro mutagenesis and use thereof.

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<sup>(f)</sup>U.S. Pat. No. 5,780,270 and Japanese Pat. No. 3466202 have been issued to Promega Corporation for site-directed mutagenesis. Other patents are pending.

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Part# 9PIM421

Printed in USA. Revised 5/04

## I. Description

T4 DNA Polymerase<sup>(a)</sup> 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).

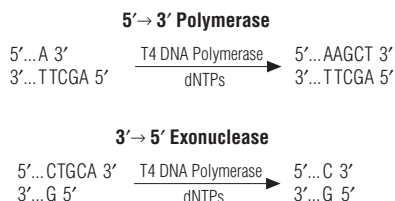


Figure 1. 5'→3' polymerase, and 3'→5' exonuclease activities of T4 DNA Polymerase.

## 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 [ $\alpha$ -<sup>32</sup>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 [ $\alpha$ -<sup>32</sup>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).

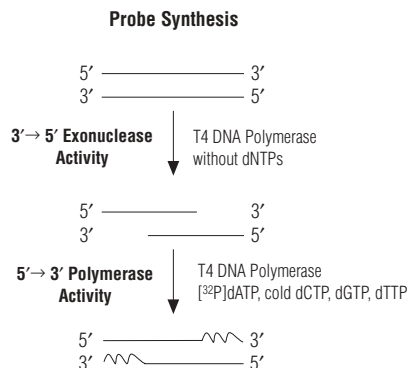


Figure 2. Probe synthesis with T4 DNA Polymerase.

## III. Related Products

Product	Cat.#
DNA Quantitation System <sup>(a,b)</sup>	K4000
Universal RiboClone <sup>®</sup> cDNA Synthesis System <sup>(a,c)</sup>	C4360
Altered Sites <sup>®</sup> II in vitro Mutagenesis System <sup>(c,d)</sup>	Q6210
Altered Sites <sup>®</sup> II-Ex1 in vitro Mutagenesis System <sup>(c,d)</sup>	Q6090
Altered Sites <sup>®</sup> II-Ex2 in vitro Mutagenesis System <sup>(c,d)</sup>	Q6080
Altered Sites <sup>®</sup> II Mammalian in vitro Mutagenesis System <sup>(c,d,e)</sup>	Q5590
GeneEditor <sup>™</sup> in vitro Site-Directed Mutagenesis System <sup>(a,f)</sup>	Q9280

<sup>a</sup>For Laboratory Use.

## IV. References

1. Challberg, M.D. and Englund, P.T. (1980) Specific labeling of 3' termini with T4 DNA polymerase. *Meth. Enzymol.* **65**, 39–43.
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.
3. Ausubel, F.M. *et al.* (1993) *Current Protocols in Molecular Biology*, Vol. 2, Greene Publishing Associates, Inc., and John Wiley and Sons, N.Y.
4. Sambrook, J. Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
5. O'Farrell, P.H., Kutter, E. and Nakanishi, M. (1980) A restriction map of the bacteriophage T4 genome. *Mol. Gen. Genet.* **179**, 421–35.