Certificate of Analysis

Part# 9PIM180

Revised 4/18

T4 DNA Ligase:

Part No.	Size (Weiss units)
M180A	100
M180B	500
M179A	(High Conc.) 500

Ligase Buffer, 10X (C126A, C126B): The Ligase 10X Buffer supplied with this enzyme has a composition of 300mM Tris-HCl (pH 7.8), 100mM $MgCl_2$, 100mM DTT and 10mM ATP. The performance of this buffer depends on the integrity of the ATP. **Store the buffer in small aliquots at –20°C to minimize degradation of the ATP and DTT**.

Note: The DTT in the Ligase 10X Buffer may precipitate upon freezing. If this occurs, vortex the buffer until the precipitate is in solution (typically 1–2 minutes). The performance of the product is not affected provided that the precipitate is resuspended.

Enzyme Storage Buffer: T4 DNA Ligase is supplied in 10mM Tris-HCl (pH 7.4), 50mM KCl, 1mM DTT, 0.1mM EDTA and 50% glycerol.

Source: *E. coli* strain expressing a recombinant clone.

Unit Definition: 0.01 Weiss unit of T4 DNA Ligase is defined as the amount of enzyme required to catalyze the ligation of greater than 95% of the *Hin*d III fragments of 1µg of Lambda DNA at 16°C in 20 minutes. See the unit concentration on the Product Information Label.

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.

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Promega

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Quality Control Assays

Activity Assays

Blue/White Assay: pGEM®-3Zf(+) Vector is digested with representative restriction enzymes (leaving 5´-termini, 3´-termini or blunt ends). Each microgram of cut plasmid is ligated with 4 units of T4 DNA Ligase. The DNA is then transformed into JM109 cells that are plated on X-Gal/IPTG/Ampicillin plates. White colonies result from transformation with ligated plasmids with damaged ends. These white colonies represent the number of false positives expected in a typical cloning experiment. Enzymes that generate overhangs must produce fewer than 2% white colonies, and blunt-cutting enzymes must produce fewer than 5% white colonies.

Contaminant Activity

Endonuclease Assay: To test for endonuclease activity, 1µg of Type I supercoiled plasmid DNA is incubated with 20 units of T4 DNA Ligase in 1X Ligase Buffer (Stock# C126 at 1X) for 16 hours at 37°C. Following incubation, the supercoiled DNA is visualized on an ethidium bromide-stained agarose gel. There must be no visible nicking or cutting of the DNA.

Single-Stranded and Double-Stranded DNase Assay: To test for DNase activity, 50ng of radiolabeled single-stranded or double-stranded DNA is incubated with 20 units of T4 DNA Ligase in 1X Ligase Buffer (Stock# C126 at 1X) for 16 hours at 37°C. Minimum passing specification is <2% release of single-stranded and <1% release of double-stranded radiolabeled nucleotides as monitored by scintillation counting of TCA-soluble material.

RNase Assay: To test for RNase activity, 50ng of radiolabeled RNA is incubated with 20 units of T4 DNA Ligase in 1X Ligase Buffer (Stock# C126 at 1X) for 5 hours at 37°C. Minimum passing specification is <3% release of radiolabeled nucleotides as monitored by scintillation counting of TCA-soluble material.

Physical Purity: The purity is ≥90% as judged by SDS-polyacrylamide gels with Coomassie® blue staining.

Signed by:

R. Wheeler, Quality Assurance

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Usage Information

I. Description

T4 DNA Ligase catalyzes the joining of two strands of DNA between the 5´-phosphate and the 3´-hydroxyl groups of adjacent nucleotides in either a cohesive-ended or blunt-ended configuration (1). The enzyme has also been shown to catalyze the joining of RNA to either a DNA or RNA strand in a duplex molecule but will not join single-stranded nucleic acids (1).

II. Standard Applications

A. Ligation of DNA

Material to Be Supplied by the User

- Nuclease-Free Water (Cat.# P1193)

We recommend using a 1:1, 1:3 or 3:1 molar ratio of vector:insert DNA when cloning a fragment into a plasmid vector. These ratios will vary with other types of vectors, for example, cDNA and genomic cloning vectors. The following example illustrates the conversion of molar ratios to mass ratios for a 3.0kb plasmid and a 0.5kb insert DNA fragment.

$$\frac{\text{ng of vector} \times \text{kb size of insert}}{\text{kb size of vector}} \times \text{molar ratio of } \frac{\text{insert}}{\text{vector}} = \text{ng of insert}$$

Example:

How much 0.5kb insert DNA should be added to a ligation in which 100ng of 3kb vector will be used? The desired vector:insert ratio will be 1:3.

$$\frac{100\text{ng vector} \times 0.5\text{kb insert}}{3\text{kb vector}} \times \frac{3}{1} = 50\text{ng insert}$$

The following ligation reaction of a 3kb vector and a 0.5kb insert DNA uses a 1:1 vector:insert ratio. Typical ligation reactions use 100–200ng of vector DNA.

1. Assemble the following reaction in a sterile microcentrifuge tube:

vector DNA	100ng
insert DNA	17ng
Ligase 10X Buffer	1µl
T4 DNA Ligase (Weiss units)	0.1–1u
Nuclease-Free Water to final volume of	10µl

2. Incubate the reaction at:

   room temperature for 3 hours, or
   4°C overnight, or
   15°C for 4–18 hours.

Notes:

1. There is considerable latitude in the temperature and time needed for successful ligations. The optimal temperature for a ligation is a balance between the optimal temperature for T4 DNA Ligase enzyme activity (25°C) (1) and the temperature necessary to ensure annealing of the fragment ends, which can vary with the length and base composition of the overhangs. Shorter duplexes (linkers less than 16 bases long) require lower temperatures as a result of their lower melting temperatures (T_m). In general, ligation reactions performed at lower temperatures require longer incubation times. The scientific literature reflects this variability in ligation conditions. Blunt-end ligations generally are efficient at temperatures between 15–20°C for 4–18 hours, while sticky ends are ligated effectively at room temperature (22°C) for 3 hours or 4–8°C overnight.
2. The ligation conditions given in this protocol are based on the conditions used at Promega for quality control of lambda vectors with sticky ends. These ligation conditions have been developed using Promega Blue/White Cloning-Qualified T4 DNA Ligase.
3. The addition of polyethylene glycol (PEG) to ligation reactions can promote ligation of blunt-ended fragments by "macromolecular crowding" (2). We do not recommend the use of PEG in ligations, however, due to extreme variability in the quality of PEG. In addition, the use of PEG can lead to undesirable concatemerization when cloning cDNAs, and residual PEG is inhibitory to lambda packaging reactions.

III. Additional Information

Molecular Weight: 68kDa (3).
Requirements: Mg^{2+}, ATP and DTT (3). The optimum concentration of Mg^{2+} is 10mM. Mn^{2+} may be substituted for Mg^{2+} but is only 25% as effective as Mg^{2+} (1).
Inhibition: 50% inhibition by greater than 150mM NaCl (activity measured at nicks) (3). Other inhibitors include 0.2M K^+, Cs^+, Li^+, NH_4^+ and 1mM spermine (1).
Inactivation: Heat to 70°C for 10 minutes (4).

IV. References

1. Engler, M.J. and Richardson, C.C. (1982) In: *The Enzymes*, Boyer, P.D., ed., Academic Press, New York, NY.
2. Zimmerman, S.B. and Pheiffer, B.H. (1983) Macromolecular crowding allows blunt-end ligation by DNA ligases from rat liver or *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **80**, 5852–6.
3. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual,* 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
4. *Protocols and Applications Guide*, Third Edition (1996) Promega Corporation.

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