Certificate of Analysis

LigaFast™ Rapid DNA Ligation System

Cat. No.	Size (Weiss units)	Size (reactions)
M8221	100	30
M8225	500	150
M8226	50	15

Description: The LigaFast™ Rapid DNA Ligation System is designed for the efficient ligation of cohesive-ended DNA inserts into plasmid vectors in just 5 minutes (blunt-ended inserts in as little as 15 minutes). Rapid ligation is based on the combination of T4 DNA Ligase with a unique 2X Rapid Ligation Buffer. The LigaFast™ System is designed to eliminate any further purification prior to transformation of ligated DNA. The specially formulated 2X Rapid Ligation Buffer requires no ATP or Mg^{2+} addition prior to use.

2X Rapid Ligation Buffer (C671B): The 2X Rapid Ligation Buffer supplied with this enzyme has a composition of 60mM Tris-HCl (pH 7.8), 20mM $MgCl_2$, 20mM DTT, 2mM ATP and 10% PEG. The performance of this buffer depends on the integrity of the ATP. Thaw the 2X Rapid Ligation Buffer at room temperature. The DTT in the Rapid Ligation Buffer may precipitate upon freezing. If this occurs, vortex the buffer until the precipitate is in solution (typically 1–2 minutes). Performance is not affected provided the precipitate is resuspended.

Enzyme Storage Buffer: T4 DNA Ligase is supplied in 10mM Tris-HCl (pH 7.0), 50mM KCl, 1mM DTT, 0.1mM EDTA, 50% glycerol.

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

Storage Conditions: 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: 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 HindIII fragments of 1µg of Lambda DNA at 16°C in 20 minutes. See the unit concentration on the Product Information Label.

Quality Control Assays

Activity Assays

Blue/White Cloning Assay: This assay is performed to demonstrate that T4 DNA Ligase is free from contaminating activities that can affect the efficiency and integrity of plasmid cloning. Any exonuclease or polymerase activity that alters the termini of linearized plasmids during ligation will result in a proportion of white-colored colonies above background levels. A pGEM® Vector is linearized with three different restriction enzymes, in separate reactions, to generate three different types of termini: 5 -overhangs, 3 -overhangs, and blunt ends. Linearized plasmids are purified using the Wizard® DNA Clean-Up System, and ligations are performed in 1X Rapid Ligation Buffer using 12 units of T4 DNA Ligase. Ligation reactions are incubated at 25°C for 5 minutes for cohesive-ended ligation or for 15 minutes for blunt-ended ligation. Competent JM109 cells are transformed with ligated plasmids and plated on X-Gal/IPTG/Amp_{100} 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. The minimum transformation efficiency must be 1×10^5cfu/µg DNA for the uncut plasmid.

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 Rapid Ligation Buffer 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.

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

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

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 Rapid Ligation Buffer 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.

Signed by:

R. Wheeler, Quality Assurance

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Usage Information

1. Standard Application

A. Ligation of DNA

Material to Be Supplied by the User

Nuclease-Free Water (Cat.# P1193)

We recommend starting with a 1:2 molar ratio of vector:insert DNA when cloning a fragment into a plasmid vector. This ratio 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 1:2.

$$\frac{100\text{ng vector} \times 0.5\text{kb insert}}{3\text{kb vector}} \times \frac{2}{1} = 33.3\text{ng insert}$$

The following ligation reaction of a 3kb vector and a 0.5kb insert DNA uses a 1:2 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	33ng
2X Rapid Ligation Buffer	5µl
T4 DNA Ligase (Weiss units)	3u
Nuclease-Free Water to final volume of	10µl

2. Incubate the reaction at room temperature for 5 minutes for cohesive-ended ligations, or 15 minutes for blunt-ended ligations.

Notes:

1. Ligation reactions performed using the 2X Rapid Ligation Buffer do not need to be cleaned up before transformation.
2. Concatamers may form as ligation products. The extent of concatamer formation depends on the vector:insert ratio, incubation temperature and incubation time (1). This should be taken into account when screening transformants.

2. Additional Information—T4 DNA Ligase

Molecular Weight: 68kDa (2).

Requirements: Mg^{2+}, ATP and DTT (2). The optimum concentration of Mg^{2+} is 0.5–1mM. Mn^{2+} may be substituted for Mg^{2+} but is only 25% as effective as Mg^{2+} (3).

Inhibition: 50% inhibition by greater than 150mM NaCl (activity measured at nicks) (2). Other inhibitors include 0.2M K^+, Cs^+, Li^+, NH_4^+ and 1mM spermine (3).

Inactivation: Heat at 70°C for 10 minutes (4).

3. References

1. Pheiffer, B.H. and Zimmerman, S.B. (1983) Polymer stimulated ligation: Enhanced blunt- or cohesive-end ligation of DNA or deoxyribooligonucleotides by T4 DNA ligase in polymer solutions. *Nucl. Acids Res.* **11**, 7853–71.
2. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual,* Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
3. Engler, M.J. and Richardson, C.C. (1982) In: *The Enzymes*, Boyer, P.D., ed., Academic Press, New York, NY.
4. *Protocols and Applications Guide*, Third Edition (1996) Promega Corporation.

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