Canvax Reagents, S.L.U.
Luis de Mercado Street, 19
Boecillo Technological Park
47151, Boecillo
Valladolid, Spain.

Tlf: +34 983 54 85 63
info@canvaxbiotech.com

www.canvaxbiotech.com

DATA SHEET

Version: 03
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1. Identification

Product name **T4 DNA Ligase**
(5 U/µL) 1000U

Cat No **CL006**

2. Description

T4 DNA ligase catalyses the formation of a phosphodiester bond between juxtaposed 5´-phosphate and 3´-hydroxyl termini in duplex DNA or RNA. This enzyme will join blunt end and cohesive end termini as well as repair single stranded nicks in duplex DNA, RNA or DNA/RNA hybrids but has no activity on single stranded nucleic acids. The enzyme requires ATP as a cofactor.

3. Composition

Item	Quantity
T4 DNA Ligase * 5 Weiss Units/µL	200 µl
10X Ligation Buffer**	1.5 ml

*Storage Buffer for T4 DNA Ligase: 20 mM Tris-HCl (pH 7.5), 50 mM KCl, 1mM DTT, 0.1 mM EDTA and 50% glycerol.
**10X T4 DNA Ligation Buffer: 400 mM Tris-HCl, 100 mM MgCl2, 100 mM DTT, 5 mM ATP (pH 7.8 at 25°C).

4. Storage

Storage at -20°C upon receipt. Avoid repeated freezing and thawing.

5. Applications

- Cloning of restriction enzyme generated DNA fragments.
- Cloning of PCR products.
- Joining of double-stranded oligonucleotides linkers or adaptors to DNA.
- Site-direct mutagenesis. • Amplified fragment length polymorphism.
- Ligase-mediated RNA detection.
- Nick repair in duplex DNA, RNA or DNA/RNA hybrids.
- Self-circularization of linear DNA.

6. Quality Control

Canvax T4 DNA Ligase has passed the following quality control assays:

- Functional absence of endonuclease and exonuclease activities.
- Blue/white cloning assay.

7. Further information

Product Use Limitations This product is developed, designed, and sold exclusively only for researchpurposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Disclaimer The information provided in this Data Sheet is correct to the best of our knowledge and belief at the date of publication. This information is intended only as a guide and should not be taken as a warranty or quality specification. **Canvax Reagents S.L.U**. shall not be held liable for any damage resulting from handling or from contact with the above product.

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Recommended Protocol

Before use:

1. Is strongly advice make aliquots of T4 DNA Ligase, and 10X T4 DNA Ligation Buffer for avoid contaminations with nucleases.
2. Spin the T4 DNA Ligase for a few seconds before use.
3. This 10X T4 DNA Ligation Buffer contains ATP, which degrades during temperature fluctuations. It is frequent to see a white precipitate on 10X T4 DNA Ligation Buffer. This precipitate buffer could be used without loss of performance. Do not try to heat the precipitate as the ATP will be degraded.

Protocol

1. **Prepare the following mixture:**
   - 10-100 ng pre-cut DNA Vector with non-compatible cohesive ends [or DNA Vector whose blunt ends could not be ligated (ex. pSpark® vectors)].
   - x ng of insert (molar ratio of vector to insert is usually from 1:1 to 1:5. **See below**).
   - 2 µL 10X T4 DNA Ligation Buffer • 1µl T4 DNA Ligase (5 U/ µL).
   - (OPTIONAL) 2 µL 50% PEG 4000 [Only Blunt Ends Ligation Protocol] [reagent not supplied]
   - up to 20 µL water nuclease-free.
2. **Incubate for 30-60 min. at 22° C.** (The overall number of colonies may be increased when reaction time is prolonged to 60 minutes and after that no additional benefit is reached).
3. **Heat inactivates at 70°C for 5 min. (Optional step that can be made only if no PEG 4000 is included in ligation)**

EXTREMELY IMPORTANT: Do not heat inactivate ligation with PEG because it reduces transformation efficiency. Alternatively, you may replace the heat inactivation by chemical inactivation with NaCl or KCl at concentrations higher than 200mM. It is possible to use the mix ligation without inactivation.

4. Use 5 to 10 µL of the mixture for transformation of 50 µL of chemically competent cells.

ADVICE: It is advised to follow the next guidelines:

1. Do not exceed the recommended amount of T4 DNA Ligase in the reaction mixture.
2. For efficient transformation, the volume of the ligation reaction mixture should not exceed 10-20% of the competent cell volume.
3. DNA resolved on agarose gel is generally visualized by illumination with ultraviolet light. Exposure to short wavelength light (e.g., 254, 302, or 312 nm) for 2 minutes reduces the ligation efficiency of DNA up to 10.000 times due to formation of pyrimidine dimmers. We strongly recommended the use of a long wavelength lamp (e.g. 360 nm) and the shortest exposure times when isolating DNA from agarose gels for cloning.
4. Polyethylene glycol (PEG) greatly increases the ligation efficiency of blunt-end DNA ligation.

Example of calculation of amount of insert:

Vector to insert molar ratios for a succesful ligation may be selected from less than 1:1 to more than 1:5 but the above ratios are the most commonly used. If you choose molar ratio of vector to insert of 1:5, use the following equation:

$$\text{ng of insert} = \frac{\text{bp insert x ng vector}}{\text{bp vector}} \text{ x molar ratio (vector to insert)}$$

Then,

$$\text{ng of insert} = \frac{\text{bp insert x ng vector}}{\text{bp vector}} \text{ x } 5$$

EXTREMELY IMPORTANT: If the ligation reaction mixture will be used for electroporation, a DNA purification step is needed before electroporation to prevent arcing of E. coli cells due to salts contained in the ligation buffer. In this case replace the heat inactivation step with spin column purification or chloroform extraction