

Certificate of Analysis

Product: T4 DNA Ligase

Code: MB-106-0100

Lot # 18735

Size: 100 units

Source: Bacteriophage T4 in *E.coli* C600 pCl857 pPLc28 lig8

Unit Definition: One unit will catalyze the exchange of one nanomole ³²-P from pyrophosphate into a Norit-absorbable compound in 20 minutes at 37° C (Weiss, B., et al., *JBC* **243**, 4543 (1968)).

Unit Conversion: One Weiss unit equals 67 Cohesive End Ligation Units. Equivalently one Cohesive End Ligation Unit equals 0.015 Weiss (ATP-PP exchange) units. A Cohesive End Ligation Unit is defined as the amount of enzyme required to give 50% ligation of Hind III digested λ DNA in 30 minutes at 16°C in 20 µl at a 5' termini concentration of 0.12 µM (300 µg/ml).

Physical State: Liquid

Enzyme Concentration: 6.0 units/µl

Storage Buffer Composition: A solution in 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 50 mM KCl and 50% (v/v) glycerol, pH 7.4.

Application(s): Cloning of restriction fragments. Joining linkers and adapters to blunt ended DNA. Catalyzes the joining of DNA fragments by forming phosphodiester bonds between juxtaposed 5' phosphate and 3' hydroxyl termini. 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. Reaction requires ATP and Mg²⁺. Optimum pH for enzymatic catalysis is 7.5.

Reagent Supplied with Enzyme: 10X T4 DNA Ligase Reaction Buffer

1X T4 DNA Ligase Reaction Buffer: 50 mM Tris-HCl, 10 mM MgCl₂, 10 mM DTT, 1 mM ATP, pH 7.5 @ 25°C.

Cofactors

ATP (included in supplied reaction buffer) is an essential cofactor for the reaction. This contrasts with *E. coli* DNA Ligase that requires NAD.

Reaction Conditions: Dilute the supplied 10X Enzyme Ligase Reaction Buffer appropriately with 9 parts Molecular Biology Grade water. Recommended DNA concentration 0.1 to 1 µM in 5' termini. **Optimal** ligation occurs at 16°C.

Room Temperature Ligation: For convenience, ligation may be done at room temperature (20–25°C). For cohesive (sticky) ends, use 1 µl of T4 DNA Ligase in a 20 µl reaction for 10 min. For blunt ends, use 1 µl of T4 DNA Ligase in a 20 µl reaction for 2 h or 1 µl high concentration T4 DNA Ligase for 10 min.

Storage Conditions: Store vials (enzyme and buffer) at –20° C or colder. Expiration date is six (6) months from date of opening.

Thawing Prior to Use: We recommend thawing 10X T4 DNA Ligase Reaction Buffer on the bench or in the palm of your hand and not at 37°C. (Thawing this buffer at 37° or higher will allow the breakdown of ATP). Once thawed, the buffer should be placed on ice.

Heat Inactivation: T4 DNA Ligase (prior to dilution) can be inactivated by incubation at 65°C for 10 min. Do not heat the DNA Ligase Reaction Buffer to 65°C (see above).

Dilution of T4 DNA Ligase: Use the formulation for Storage Buffer Composition, as stated above, to dilute enzyme for –20°C storage. To dilute for immediate use, 1X T4 DNA Ligase Reaction Buffer can be used.

Other Enzymatic Activity: DNase – none detected after incubation of 2 µg Lambda DNA with 10 units of T4 DNA Ligase for 1 hour at 37° C in a total volume of 100 µl. Endonuclease – No change in the ethidium bromide electrophoresis band pattern following the incubation of 10 units of T4 DNA Ligase with 2 µg of pUC18 DNA. RNase – None detected after incubation of 20 µg of rRNA with 10 units of T4 DNA Ligase for 2 hours at 37° C in a total volume of 20 µl.

References:

Engler, M.J. and Richardson, C.C. (1982) in *The Enzymes* (Boyer, P.D., ed.) Vol. 5, p. 3, Academic Press, San Diego.

Sambrook, J. et al. (1989) *Molecular Cloning: A Laboratory Manual*, second edition, pp.1.53–1.73, Cold Spring Harbor, New York.

Weiss, B., et al. (1968) *J. Biol. Chem.* **243**, 4543–4555.

USDA Certification: All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation.

Note: This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information.