T4 DNA Ligase

Catalogue No	202-1, 500 Wu 202-2, 3x500 Wu
Concentration	2.5 Wu/µl and 6 Wu/µl*

**Add an H to cat.# to order the high concentration*

Reagents supplied: 10x Ligase Reaction buffer (w/o ATP).

Source: T4 DNA ligase is purified from *E. coli* lambda lysogen NM 989.

Description: T4 DNA Ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5'-phosphate and 3'-hydroxyl termini in duplex DNA or RNA.

Unit definition: One Weiss unit is defined as the amount of enzyme required to catalyze the exchange of 1 nmol of ^{32}P from pyrophosphate to ATP, into Norit-adsorbable material in 20 minutes at 37°C

Reaction conditions: 50 mM Tris-HCl (pH 7.8), 10 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM ATP (not included) and DNA (recommended DNA concentration 0.1 to 1 µM of 5´ termini). Optimal ligation occurs at 16°C.

Quality control: Tested for the absence of endo- and exodeoxyribonucleases, ribonucleases and for the capacity to join cohesive- and blunt-ended DNA fragments.

Storage buffer: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol. Store at -20°C.

Heat inactivation: T4 DNA Ligase can be inactivated by incubation at 65°C for 10 minutes.

Notes

• One Weiss unit is equivalent to circa 67 cohesive-end ligation units.

• T4 DNA Ligase is strongly inhibited by NaCl or KCl if the concentration exceeds 200 mM.

• Ligation of blunt-ended and single-base pair overhang fragments requires about 50 times as much enzyme to achieve the same extent of ligation as cohesive-end DNA fragments. Blunt-end ligation may be enhanced by addition of PEG or hexamine chloride, or by reducing the ATP concentration to 50 µM.

Recommended ligation mixtures:

- Sticky-end ligation mixture

T4 DNA ligase	2.5-6Wu
10x Ligase buffer	2µl
10mM ATP	2µl
Linear DNA vector	50-100ng
DNA insert	1:1-1:5 (vector:insert)
Sterile ultrapure water	Up to 20 µl

Incubate overnight at 16°C or for 30min at 25°C

- Blunt-end ligation mixture

T4 DNA ligase	6Wu
10x Ligase buffer	2µl
10mM ATP	0.1-2µl
50% w/v PEG 4000	2µl
Linear DNA vector	50-100ng
DNA insert	1:1-1:5 (vector:insert)
Sterile ultrapure water	Up to 20 µl

Incubate overnight at 16°C or for 2h at 25°C