LaboPass™ T4 DNA Ligase

Contents	Conc.	CMX0101	CMX0105
T4 DNA Ligase	400 U/μl	20,000 U	100,000 U
10X T4 DNA Ligase Buffer		1 ml	1 ml x 2 vials

Description

The LaboPass™ T4 DNA ligase is isolated from a recombinant source. This enzyme catalyzes the formation of a phosphodiester bond between neighboring 5′ phosphate and 3′ hydroxyl termini of double-stranded DNA in either a cohesive or blunt-ended configuration. Single strand break in dsDNA is repaired by T4 DNA ligase. T4 DNA ligase can also catalyze the ligation of RNA to duplex DNA or RNA, but the activity toward single stranded DNA or RNA is very low.

Applications

- Cohesive or blunt termini ligation
- Repair of nicks in duplex DNA or RNA
- Linker or adaptor joining to DNA fragments

Unit definition

Labopass™ T4 DNA Ligase is defined in cohesive end ligation unit. One unit is defined as the amount of enzyme required to give 50% ligation of HindIII fragments of Lambda DNA in 20 μl of 1X T4 DNA Ligase Reaction Buffer in 30 minutes at 16℃. One cohesive end ligation unit is 0.015 Weiss unit approximately.

10X T4 DNA Ligase Buffer

660 mM Tris-HCl pH7.5, 50 mM $MgCl_2$, 10 mM ATP and 50 mM DTT.

* Store the buffer in small aliquots at -20℃ to minimize degradation of the ATP and DTT.

DTT may precipitate at low temperature. Vortex until it is clear of precipitates before using.

Storage Buffer

10 mM Tris-HCl pH7.4, 50 mM KCl, 0.1mM EDTA, 1 mM DTT and 50% glycerol

Quality Control

Nuclease activity test

0.5 μg of supercoiled pUC19, λ DNA, and λ DNA/HindIII DNA are incubated with 20 units of T4 DNA Ligase in 1X Ligase Buffer for 10 hours at 37℃. Following incubation, nicking, cutting or smearing of the DNA is not detected on an agarose gels.

The protein purity

As judged by Coomassie blue staining of SDS-PAGE, the enzyme purity is more than 95%.

Standard reaction protocol for gene cloning

1. Mix the following reaction components in a sterile microfuge tube and briefly spin-down

10X T4 DNA Ligase Buffer	1 μl
Vector DNA	x μl (20-200 ng)
Insert DNA	y μl*
T4 DNA Ligase (400 U/μl)	0.5-1 μl
Nuclease-free wate	

* Normally a vector to insert ratio of 1 to 3 is used of cohesive end ligations. Higher molar ratios can be used for blunt end ligations. The amount of insert DNA to be used in the reaction can be calculated by the following formula

$$\text{Insert Mass (ng)} = \left[\text{molar ratio of } \frac{\text{insert}}{\text{vector}} \right] \times \left[\frac{\text{insert length (bp)}}{\text{vector length (bp)}} \right] \times \text{Vector Mass (ng)}$$

2. Incubate for either 0.5-2 hours at room temperature, or 16℃ overnight.
3. Heat inactivate at 65℃ for 10 minutes (optional).
4. Proceed to the transformation of E. coli.

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