Yeastern Biotech Co., Ltd

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YB Rapid Ligation Kit

Cat. No.
FYC003-100R

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YB Rapid Ligation Kit

Concentration: 3 U/μl **Storage:** - 20 °C

Description

Yeastern's yT4 DNA ligase catalyzes the joining of two strands of DNA between the 5'–phosphate and the 3'–hydroxyl groups of adjacent nucleotides in either a cohesive-ended or blunt-ended termini. The enzyme also repairs single-strand nicks in duplex DNA, RNA or DNA/RNA hybrids. YB Rapid Ligation Kit is designed for efficient ligation of DNA inserts into vectors in just 5 minutes.

Component	Concentration	Volume
yT4 DNA ligase	3 U/μl	100 μl
10X Ligation Buffer A		200 μl
10X Ligation Buffer B		200 μl

yT4 DNA ligase Storage Buffer:

20 mM Tris-HCl (pH 7.5), 50 mM KCl, 1 mM DTT, 0.1 mM EDTA and 50% glycerol.

10X Ligase Buffer A:

0.4 M Tris-HCl, 0.1 mM $MgCl_2$, 0.1 M DTT and 5 mM ATP (pH 5.0 at 25°C).

10X Ligase Buffer B:

A buffer contains an enhancer which dramatically increases ligation efficiency for blunt end DNA.

Unit Definition:

One unit of enzyme catalyzes the conversion of 1 nanomole of [^{32}PPi] into Norit-adsorable form in 20 min at 37°C (Weiss unit).

Standard Applications

- Joining double-stranded DNA with cohesive or blunt termini.
- Joining oligonucleotide linkers to blunt-ended DNA.
- Repairing nicks in duplex DNA, RNA or DNA-RNA hybrids.

Procedure

1. In a microcentrifuge tube prepare 5-10 μl mix in ddH_2O or TE buffer of digested vector DNA and foreign DNA to be inserted.

2. Add the following components to the same tube:

	Cohesive Ends	Blunt Ends
vector: insert molar ratio	1 : 3	1 : 6
Vector fragments end conc.	3-30 fmol	15-60 fmol
Insert fragments end conc.	9-90 fmol	45-180 fmol
10X Ligation Buffer A	2 μl	2 μl
10X Ligation Buffer B	2 μl	2 μl
yT4 DNA ligase	1 μl	1 μl
ddH_2O to final volume of	20 μl	20 μl

3. Vortex the tube and spin down in microcentrifuge for 3-5 sec.
4. Incubate the mixture for 5-20 min. at 22°C.
5. If the insert fragment size >3 kb, incubate the ligation mixture at 4°C overnight.
6. Use the mixture for transformation.

Note

1. yT4 DNA ligase is strongly inhibited by NaCl or KCl if the concentration exceeds 0.2 M.
2. 10X Ligation Buffer B greatly increase the rate of ligation of blunt-ended DNA.
3. Use equal or higher (up to 3-fold) molar concentration of insert DNA over vector DNA.
4. If the yield of ligation product is insufficient, prolong the reaction time (overnight).
5. Ligation reactions performed at lower temperatures require longer incubation time.
6. The performance of Ligation buffer A depends on the integrity of the ATP. Store the buffer in small aliquots at -20°C to minimize degradation of the ATP and DTT.
7. The DTT in the 10X Ligation Buffer A may precipitate upon freezing. If this occurs, vortex the buffer until the precipitate is completely dissolved in solution.
8. yT4 DNA Ligase is unstable on ice for a long period of time, please do not put it out of -20°C longer than 5-10 min.

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