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RBC Rapid Ligation Kit

Rapid and Economical Ligation

Store at -20°C

Cat.No. **RC011** (100 reactions)

T4 DNA Ligase (3 U/µl):100 µl
10X Ligation Buffer A: 200 µl
10X Ligation Buffer B: 200 µl

Description

T4 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. RBC Rapid Ligation Kit is designed for efficient ligation of DNA inserts into plasmid vectors in just 5 minutes.

Unit Definition

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

Applications

Joining double-stranded DNA with sticky or blunt termini.

Joining of oligonucleotide linkers to blunt-ended DNA.

Repairing nicks in duplex DNA, RNA or DNA-RNA hybrids.

Quality Control

Nuclease activity is not detected after incubation 1 µg lambda/Hind III DNA with 5 units RealHi DNA Polymerase in 50 µl reaction volume in supplied Reaction Buffer for 18 hours at 37°C.

Storage Conditions

RBC Rapid Ligation Kit should be stored immediately upon receipt at -20°C in a constant temperature freezer. RBC Rapid Ligation Kit can be stored for up to 12 months without showing any deduction in performance and quality with proper storage.

Storage Buffer

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

10X Ligation 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). The performance of this buffer depends on the integrity of the ATP. Store the buffer in small aliquots at –20°C to minimize degradation of the ATP and DTT.

10X Ligation Buffer B

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

Things Before Starting:

1. The DTT in the 10X Ligation Buffers may precipitate upon freezing. If this occurs, vortex the buffer until the precipitate is in solution. The performance of the product is not affected if the precipitate is resuspended.
2. The 10X Ligation Buffers are supplied as 10X concentrate and should be diluted for use.

Standard Protocol

1. We recommend using a 1:3 molar ratio of vector: insert DNA when cloning a fragment into a plasmid vector. These ratios will vary with other types of vectors.
2. In a microcentrifuge tube prepare 5-10 µl mix in water or TE buffer of digested vector DNA (50-400 ng) and foreign DNA to be inserted.
3. Add the following components to the same tube:
   - i. 10X Ligation Buffer A 2 µl
   - ii. 10X Ligation Buffer B 2 µl
   - iii. T4 DNA Ligase 1 µl
   - iv. Nuclease-Free Water to final volume of 20 µl.
4. Vortex the tube and spin down in microcentrifuge for 3-5 seconds.
5. Incubate the mixture for 5 -20 minutes at 22°C.
6. Inactivate T4 DNA Ligase by heating the reaction mixture at 65°C for 10 minutes. Use the mixture for transformation.

Note

1. For research use only. Not for use in diagnostic or therapeutic procedures.
2. T4 DNA Ligase is strongly inhibited by NaCl or KCl if the concentration exceeds 0.2 M.
3. 10X Ligation Buffer B greatly increases the rate of ligation of blunt-ended DNA.
4. The inactivation of T4 DNA Ligase by heating at 65°C for 10 minutes is recommended as a standard procedure prior to transformation of cells with DNA. In some cases this simple step can increase the number of transformants by two orders of magnitude.
5. It is necessary to extract DNA prior to transformation.
6. Use equal or higher (up to 3-fold) molar concentration of insert DNA termini over vector DNA.
7. If the yield of ligation product is insufficient, prolong the reaction time (overnight).
8. Ligation reactions performed at lower temperatures require longer incubation times.

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