T4 DNA Ligase (Rapid)

Catalog # N103

Version 6.1

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Introduction

The T4 DNA Ligase catalyzes the formation of a phosphodiester bond between the adjacent 5'-phosphate and 3'-hydroxyl on the blunt or cohesive end of dsDNA. This enzyme will join blunt end and cohesive end termini as well as repair single-stranded nicks in double-stranded DNA, RNA, and DNA/RNA hybrids.

Package Information

Components	N103-01 60,000 U
T4 DNA Ligase (Rapid) (600 U/μl)	1 ml
2× Rapid Ligation Buffer	6 ml
10× T4 DNA Ligase Buffer	2 ml

Buffer Contents

2× Rapid Ligation Buffer

132 mM Tris-HCl pH 7.6 @ 25℃
20 mM $MgCl_2$
2 mM DTT
2 mM ATP
15% PEG 6000

10× T4 DNA Ligase Buffer

500 mM Tris-HCl pH 7.6 @ 25℃
100 mM $MgCl_2$
50 mM DTT
10 mM ATP

Storage

Store at -20℃.

Unit Definition

In a ligation reaction system of 50 μl, one unit (U) is defined as the amount of enzyme required to catalyze the ligation of more than 50% of 100 ng λDNA-Hind III DNA fragments in 30 min at 23℃.

Protocol 1: Ligation of Insert DNA & Vector DNA

1. Prepare the following reaction solution in a microcentrifuge tube:

10× T4 DNA Ligase Buffer	1 μl
Insert DNA [a]	0.3 pmol
Vector DNA [b]	0.03 pmol
T4 DNA Ligase (Rapid) (600 U/μl)	1 μl
Sterile distilled water	to 10 μl

Note: 1. The molar ratio of Insert/Vector should be between 3: 1 and 10: 1.
2. The blunt-end vector should firstly be dephosphorylated to avoid self-cycling.

2. Incubate overnight at 16℃.

3. Transformation.

3.1. Add the ligation product to 100 μl of competent cells. The volume of the ligation product should be less than 1/6 of the volume of competent cells. Mix gently and incubate for 30 min on ice.

3.2. Incubate the mixture at 42℃ in a water bath for excatly 90 seconds. Then immediatly chill on ice for 2 min-3 min without disturbing the mixture.

3.3. Add 900 μl of LB or SOC medium to the centrifuge tube. Then out the tube in a shaker-incubuator (150 rpm, 37℃) for 45 min, during which the cells will recover and express the resistance gene.

3.4. Centrifuge at 2,500 × g for 5 min and discard 900 μl of supernatant. Resuspend the cells with the remaining medium and gently coated on a agar plate containing the appropriate antibiotics. Incubate overnight at 37℃.

Protocol 2: Adaper Ligation in DNA Library Preparation

1. Prepare the following reaction solution in a microcentrifuge tube:

dA-Tailing Products [a]	10 μl
2× Rapid Ligation Buffer	15 μl
DNA Adapter [b]	2.5 μl
T4 DNA Ligase (Rapid) (600 U/μl)	2.5 μl

Note: 1. The product is DNA fragments with phosphorylated 5 'ends and 3'-dA ends
2. The molar ratio of dA-Tailing Products/DNA Adapter should be between 1:10 and 1:20.

2. Incubate in a PCR instrument as follows:

37℃	10 min
4℃	Hold

The ligation products should be used for the next procedure immediately.