



Product code

KK6003
KK6004
KK6005
KK6006

Kit size

20,000 units (400,000 U/ml)
100,000 units (400,000 U/ml)
20,000 units (2,000,000 U/ml)
100,000 units (2,000,000 U/ml)

1. Production Description

KAPA T4 DNA ligase catalyzes the formation of a phosphodiester bond between 5' phosphate and 3' hydroxyl termini in duplex DNA or RNA. This enzyme will join blunt and cohesive end termini as well as repair single stranded nicks in duplex DNA, RNA or DNA/RNA hybrids¹.

2. Product Applications

KAPA T4 DNA Ligase is ideally suited for:

- routine subcloning
- recircularization of linear DNA
- library construction
- linker ligation

3. Ligation Protocol

3.1. In a microcentrifuge tube, combine the following reagents:

Vector DNA	x µl (10-100 ng)
Insert DNA	x µl*
10X ligation buffer	2 µl
Sterile ddH ₂ O	Up to 20 µl
KAPA T4 DNA Ligase	1 µl (400U or 2,000U)

- 3.2. Vortex the tube and briefly centrifuge.
3.3. Incubate the mixture for 1 hour at room temperature.
3.4. Immediately transform competent cells** with 2 µl of the ligation reaction.

*For cohesive-end ligations, use a 1:1 or a 3:1 molar ratio of insert:vector; for blunt-end ligations, use a 3:1 molar ratio of insert:vector DNA.

**Transformation can be done using chemically-competent or electrocompetent cells. Electrocompetent cells may show significantly higher transformation efficiency (several logs higher).

4. Basic Transformation Protocol

1. Thaw competent cells on ice.
2. Chill ~5ng ligation mix (2 µl) on ice in sterile microcentrifuge tube.
3. Add 50 µl thawed, mixed competent cells to DNA and gently mix by pipetting.
4. Incubate on ice for 30 minutes.
5. Heat shock at 42°C for 2 minutes, immediately return transformation mix to ice for 5 minutes.
6. Add SOC*** media (950 µl) to cells, mix gently, and incubate at 37°C for 1 hour.
7. Spread 100 µl onto desired plate medium.
8. Incubate overnight at 37°C.

*** SOC media: 2% Bactotryptone, 0.5% Yeast extract, 2.5mM KCl, 10mM NaCl, 10mM MgSO₄, 10mM MgCl₂ and 20mM Glucose.

5. Quality Control

SDS-PAGE

2.0 µl (10.0 µg) of enzyme solution was loaded on a denaturing 4-20% Tris-Glycine SDS-PAGE gel flanked by a broad-range MW marker and 2.0 µl (100 ng) of a 1:100 dilution of the sample. Following electrophoresis, the gel was stained using the silver stain technique (Invitrogen SilverQuest). The aggregate mass of contaminant bands in the concentrated sample did not exceed the mass of the protein of interest band in the dilute sample, confirming greater than 99% purity of the concentrated sample.

Single-Stranded Exonuclease Assay

A 50 µl reaction containing 50,000 cpm of tritiated oligo dT and 10 µl (50,000 U) of enzyme solution incubated for 16 hours at 37°C resulted in less than 0.1% release of TCA-soluble counts.

Double-Stranded Exonuclease Assay

A 50 µl reaction containing 15,000 cpm of a 1 kb, tritiated, double stranded DNA fragment and 10 µl (50,000 U) of enzyme solution incubated for 4 hours at 37°C resulted in less than 0.1% release of TCA-soluble counts.

Endonuclease Activity

A 50 µl reaction containing 1 µg of pBR322 DNA and 10 µl (50,000 U) of enzyme solution incubated for 4 hours at 37°C resulted in no visually discernible conversion to nicked circular DNA as determined by agarose gel electrophoresis.

Real-Time PCR DNA Contamination Test

Replicate 5 µl samples were heat denatured and screened in a TaqMan® qPCR assay for the presence of contaminating *E.coli* genomic DNA using primers for the 16S rRNA locus. The absolute quantification method of detection was employed, with serial dilutions of purified *E.coli* K-12 used to draw a standard curve (5 points, R²=0.991). Based on no template control Ct values, the detection limit of this assay is <10 copies genome/sample. Replicate average samples was observed to be <10 copies of genome/25,000 U T4 DNA Ligase.

Unit Characterization Assay

Unit activity was measured using a 2-fold serial dilution method. Dilutions of enzyme batch were made in 1X KAPA T4 DNA Ligase Reaction Buffer ([T4 DNA Ligase]_f = 0.31-20 µg/µl) and added to 50 µl reactions containing 0.1 µg DNA and 1X KAPA T4 DNA Ligase Reaction Buffer. Reactions were incubated 30 minutes at 23°C (room temp), plunged on ice, and analyzed on a 1% agarose gel stained with Ethidium Bromide. 1 unit is defined as the amount of KAPA T4 DNA Ligase required to ligate 50% of 100 ng DNA fragments with cohesive termini in 50 µl following a 30 minute incubation at 23°C.

6. References

1. Engler, M.J. and Richardson, C.C. (1982) P.D. Boyer (Eds.), *The Enzymes*, 5, pp. 3. San Diego: Academic Press.

7. Limitations on Use

This product was developed, manufactured, and sold for research and in vitro use only. The product is not suitable for administration to humans or animals. MSDS sheets relevant to this product are available upon request.

For more information on KAPA T4 DNA Ligase please visit www.kapabiosystems.com or contact your local representative.

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