



KAPA™ Rapid Ligation System

Technical Data Sheet

Product Code

KK6101

KK6102

Kit size

50 reactions

150 reactions

1. Production Description

The KAPA Rapid Ligation System is a combination of KAPA Rapid T4 DNA Ligase and an optimized reaction buffer designed to ligate blunt or cohesive end DNA fragments in 5 minutes at room temperature.

2. Product Applications

The KAPA Rapid Ligation System is ideally suited for:

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

3. KAPA Rapid Ligation Protocol

1. Combine 50 ng vector DNA with insert at a 3-fold molar excess.
2. Adjust volume to 10 µl with sterile water.
3. Add 10 µl 2X KAPA Rapid Ligation Buffer and mix.
4. Add 1.0 µl KAPA T4 DNA Ligase and mix gently.
5. Centrifuge briefly to bring contents to base of tube, incubate 5 minutes at room temperature.
6. Immediately transform competent cells with 2 µl of the ligation reaction*. Do NOT heat inactivate.

*Transformation can be done using chemically-competent or electrocompetent cells. Electrocompetent cells may show significantly higher transformation efficiency (several logs higher), and it is advisable to purify the KAPA Rapid Ligation reaction by performing a spin column DNA purification step.

4. Basic Transformation Protocol

The following protocol is recommended for transforming ligation products generated with the KAPA Rapid Ligation System:

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.

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Notes

- The KAPA Rapid Ligation System is designed to promote ligation in 5 minutes at room temperature. Incubations of longer than 5 minutes are unnecessary and not recommended due to a reduction in transformation efficiency.
- Heat inactivation of the KAPA Rapid Ligation mix is not recommended as it may significantly reduce transformation efficiency.
- The system and usage protocol were intended for a 20 µl reaction volume. Larger reaction volumes are acceptable but must maintain a 1X Rapid Ligation Buffer concentration and 1 µl KAPA Rapid T4 DNA Ligase per 20 µl reaction volume.
- Optimal ligation efficiency is observed when the overall vector/insert concentration is maintained at 1-10 µg/ml. Insert:vector ratios between 2 and 6 will maximize single-insertion event, while ratios below 2:1 will result in decreased ligation efficiency and above 6:1 will favor the formation of multimers. A titration with decreasing DNA concentrations may be necessary if DNA concentration is unknown.

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 (15,000 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 (15,000 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 (15,000 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, $R^2=0.991$). 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/7,500 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. 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 technical support please contact: support@kapabiosystems.com