KAPA Rapid Ligation System

KR0472 – v1.13

Product Description

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

KAPA Rapid Ligase contains T4 DNA Ligase, which 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 hybrids[1].

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

Product Applications

The KAPA Rapid Ligation System is ideally suited for:

- Routine subcloning
- Recircularization of linear DNA
- Library construction
- Linker ligation

KAPA Rapid Ligation Protocol

1. In a microcentrifuge tube, combine the following reagents for a 20 µl ligation reaction:

2X KAPA Rapid Ligation Buffer	10 µl
Vector DNA	50 ng
Insert DNA	As required*
25 U/µl KAPA Rapid Ligase**	1 µl (25 U)
Sterile ddH_2O	Up to 20 µl

2. Vortex the tube and briefly centrifuge.
3. Incubate the mixture for 5 minutes*** at room temperature.
4. Immediately transform competent cells with 2 µl of the ligation reaction.

* Use a 2:1 to 6:1 molar ratio of insert:vector DNA. Lower ratios may result in reduced ligation efficiency, while higher ratios may promote the formation of multimers. Maintain an overall vector/insert concentration of 1 – 10 µg/ml.

** KAPA Rapid Ligase is defined in Weiss units. One Weiss unit is equivalent to approximately 80 cohesive end units.

*** Incubations longer than 5 minutes are unnecessary and may lead to reduced transformation efficiency.

Kit Codes and Components	
KK6110 (50 rxns)	KAPA Rapid Ligase (25 U/µl) – 1 x 1250 U KAPA Rapid Ligation Buffer (2X) – 3 x 250 µl
KK6111 (150 rxns)	KAPA Rapid Ligase (25 U/µl) – 3 x 1250 U KAPA Rapid Ligation Buffer (2X) – 7 x 250 µl

Quick Notes

- The KAPA Rapid Ligation System performs blunt- and cohesive-end ligations in 5 minutes at room temperature.
- Use a 2:1 to 6:1 molar ratio of insert:vector, and 25 U (1 µl) of KAPA T4 Rapid DNA Ligase per 20 µl reaction.
- Use 1 µl (25 U) of KAPA Rapid Ligase per 20 µl reaction
- Heat inactivation is not recommended and may dramatically reduce transformation efficiency.

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 ~5 ng 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** medium (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.

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

** SOC medium: 2% Bacto-tryptone, 0.5% Yeast extract, 2.5 mM KCl, 10 mM NaCl, 10 mM $MgSO_4$, 10 mM $MgCl_2$ and 20 mM Glucose.

KAPA Rapid Ligation System

Technical Data Sheet

Product Specifications

Shipping and Storage

Upon arrival, store kit components at -20 °C in a constant-temperature freezer. When stored under these conditions and handled correctly, the reagents will retain full activity until the expiry date indicated on the kit label.

Handling

Always ensure that the product has been fully thawed and mixed before use.

Product Components

- KAPA Rapid Ligase is supplied in 10 mM Tris-HCl, 50 mM KCl, 1 mM Dithiothreitol, 0.1 mM EDTA, 50% Glycerol, pH 7.4 @ 25 °C
- 2X KAPA Rapid Ligation Buffer

Quality Control

SDS-PAGE

5000 U of enzyme was loaded on a denaturing 4 – 20% Tris-Glycine SDS-PAGE gel flanked by a broad-range MW marker and 2.0 µl of a 1:100 dilution (50 U) 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 2500 U of enzyme 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 2500 U of enzyme 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 2500 U of enzyme 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 create a standard curve (5 points, R^2 = 0.991). Based on no template control Cq values, the detection limit of this assay is <10 copies genome/sample. Replicate average samples was observed to be <10 copies of genome per 312.5 U KAPA Rapid Ligase.

Unit Characterization Assay

One Weiss unit is defined as the amount of protein required to catalyze the conversion of 1 nmol of [32PPi] into Norbit absorbable form in 20 min at 37 °C. One Weiss unit is equivalent to approximately 80 cohesive end ligation units.

Limitations of 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

KAPABIOSYSTEMS

Boston, Massachusetts, United States
Int. Tel: +1 781 497 2933 Tel: +1 855 KAPA BIO
Fax: +1 781 497 2934
Email: sales@kapabiosystems.com

Cape Town, South Africa
Tel: +27 21 448 8200 Fax: +27 21 448 6503
Email: sales@kapabiosystems.com