

Product Description

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

Recommended Applications

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

Source of Protein

A recombinant *E. coli* strain carrying the cloned T4 DNA Ligase gene.

Components

Component Name	NP100032	NP100033
T4 DNA Ligase (supplied in 10 mM Tris-HCl, pH 7.4, 50 mM KCl, 1 mM dithiothreitol, 0.1 mM EDTA, 50% Glycerol.)	30 μ L (2000 U/ μ L)	150 μ L (2000 U/ μ L)
2X Rapid Ligation Buffer containing ATP and PEG.	1 mL	2 x 1 mL

Storage Conditions

Store at -20°C.

Quality Assurance

Purified free of contaminating endonucleases and exonucleases. In addition, enzyme purity is analyzed by SDS-PAGE, and negligible *E.coli* genomic DNA is confirmed by qPCR.

Safety and Use Statement

All biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of potentially infectious or hazardous agents. This product is authorized for laboratory research use only. The product has not been qualified or found safe and effective for any human or animal diagnostic application. Uses other than the labeled intended use may be a violation of applicable law.

References

Engler, M .J . and Richardson, C .C . (1982) in *The Enzymes* P.D. Boyer, eds., Vol 5, p. 3, Academic Press, San Diego.

Rapid Ligation Protocol

1. To a sterile 1.5 mL tube, combine 50 ng vector DNA with insert at a 3-fold molar excess.
2. Adjust volume to 10 μ L with sterile water.
3. Add 10 μ L 2X Rapid Ligation Buffer and mix.
4. Add 1.0 μ L 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 2 μ L into competent cells. (Do **NOT** heat inactivate.)

Basic Transformation Protocol

The following protocol is recommended for transforming ligation products generated with the Rapid Ligation Kit

1. Thaw competent cells on ice.
2. Chill ~5ng ligation mix (2 μ L) on ice in sterile microcentrifuge tube.
3. Add 50 μ L 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 μ L) to cells, mix gently, and incubate at 37°C for 1 hour.
7. Spread 100 μ L of the reaction onto desired plate medium.
8. Incubate overnight at 37°C.

Heat Inactivation

Heat inactivation of the Rapid Ligation mix is **NOT** recommended as it may significantly reduce transformation efficiency.

Usage Notes

1. The Rapid Ligation Kit 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.
2. The kit and usage protocol were intended for a 20 μ L reaction volume. Larger reaction volumes are acceptable but must maintain a 1X Rapid Ligation Buffer concentration and 1 μ L T4 DNA Ligase per 20 μ L reaction volume.
3. Optimal ligation efficiency is observed when the overall vector/insert concentration is maintained at 1-10 μ g/mL. In general, a 3:1 molar excess of insert to vector is recommended. Insert to vector ratios below 2:1 will result in decreased ligation efficiency and above 6:1 will favor the formation of multimers. If your DNA concentration is unknown, perform multiple ligations using different insert to vector ratios.
4. Electrocompetent cells may show significantly higher transformation efficiency (several logs higher), and it is advisable to reduce the concentration of PEG in the Rapid Ligation reaction by purifying the ligation mixture on a spin column or by ethanol precipitation before electroporation. Alternatively, dilute the ligation mixture 5-fold in TE buffer prior to transformation and use 1 μ L of the diluted ligation reaction per 40 μ L of electrocompetent cells.