DNA Ligation Kit

FB02502005 50 Reactions
FB02502015 150 Reactions
Store at -25°C to -15°C

DNA Ligation Kit enables fast sticky-end or blunt-end DNA ligation in only 5 minutes at room temperature. The kit contains T4 DNA ligase, and a specially formulated 5X ligation buffer optimized for fast and efficient DNA ligation. Fast ligation efficiency is equal to that obtained with T4 DNA ligase in a standard 1-hour ligation. The ligation reaction mixture can be used directly for bacterial transformation.

Applications

- Routine cloning experiments
- Blunt-end cloning
- Library construction
- TA cloning

Kit Contents

Reagents	50 Reactions	150 Reactions
T4 DNA Ligase, 5 U/µL	50 µL	150 µL
5X Ligation Buffer	1 mL	2 x 0.75 mL
Water, nuclease-free	1.25 mL	2 x 1.25 mL

Protocol 1: Ligation of insert DNA into plasmid vector DNA

1. Thoroughly mix the 5X Ligation buffer prior to use.
2. Prepare the following reaction mixture:

Component	20 µL reaction
Linearized vector DNA*	10-100 ng
Insert DNA (at 3:1 molar excess over vector)	varies
5X Ligation Buffer	4 µL
T4 DNA Ligase, 5 U/µL	1 µL
Water, nuclease-free	to 20 µL

*Do not exceed the maximum recommended amount of vector DNA of 200 ng in 20 µL reaction volume.

3. Vortex and spin briefly to collect drops.
4. Incubate the mixture at 22°C for 5 min.
5. Use 2-5 µL of the ligation mixture for transformation.

Note:
The reaction mixture can be stored at 0-4°C until used for transformation. Prior to electroporation, chloroform extract the ligation mixture and use 1 µL for the electroporation reaction.

Protocol 2: Recircularization of linear DNA

1. Thoroughly mix the 5X Ligation buffer prior to use.
2. Prepare the following reaction mixture:

Component	50 µL Reaction
Linearized vector DNA	10-50 ng
5X Ligation Buffer	10 µL
T4 DNA Ligase, 5 U/µL	1 µL
Water, nuclease-free	to 50 µL

3. Vortex and spin briefly to collect drops.
4. Incubate the mixture at 22°C for 5 min.
5. Use 2-5 µL of the ligation mixture for transformation.

Note:
The reaction mixture can be stored at 0-4°C until used for transformation. Prior to electroporation, chloroform extract the ligation mixture and use 1 µL for the electroporation reaction.