



Instruction for Use

DNA Ligation Kit

AG11801

Version.V1E1

**Research Use Only
Not For Diagnosis Procedures**

1. Description

This Product is designated for accurate, fast, efficient DNA ligation experiment based on T4 DNA Ligase system. This kit is optimized for fast ligation reaction with small volume of reaction product. Output solution could directly be used for bacterium transformation without purification. Component Ligation Solution A contains T4 DNA Ligase and reaction buffer; Component Ligation Solution B contains enhancer for linear DNA ligation and adapter ligation; Component Ligation Solution C is transformation enhancer to enhance heat transformation efficiency.

2. Kit Information

Kit Name	Cat. No	Specification
DNA Ligation Kit	AG11801	100 rxns (7.5 µl/rxn)

3. Transportation and Storage

Storage	Store at -20°C
Transportation	Transport at -20°C Dry Ice or Blue Ice Condition

4. Kit Components

Kit Components	Volume
Ligation Solution A ^{*1}	250 µl x 3 pc
Ligation Solution B ^{*2}	750 µl
Ligation Solution C ^{*3}	200 µl

*1: Ligation Solution A is contained with T4 DNA Ligase. Before use, thaw in ice and mix gently. Stored at -20°C after use.

*2: Ligation Solution B should be thawed at room temperature and then mix well. Stored at -20°C after use.

*3: Ligation Solution C can be stored at room temperature after thawing. For precipitation, mix thoroughly or incubate at 37°C to dissolve, then store at room temperature for further use.

5. Protocol

5.1 Ligation of Circular DNA

Components	Volume
DNA mix *1	5-10 μ l
Ligation Solution A	Equal Volume as above

Reaction: 16°C for 30 min*2

[Reaction Product could directly be used for bacterium transformation]*3

*1: The molar ratio of Vector DNA and Insert DNA is recommended to be 0.03 pmol: 0.03 -0.3 pmol.

If Insert DNA is Linker DNA, then the molar ratio is recommended to be 1:100 or above.

*2: For unsatisfactory results, prolong the reaction time to overnight.

Incubation at 70°C for 10 min could deactivate the T4 DNA Ligase.

*3: Use Ligation Solution C to enhance the heat transformation efficiency. For example, add 1 μ l Ligation Solution C for 9 μ l of reaction solution, mix well before transformation. For electroporation (electropermeabilization), recommended to precipitate the solution with ethanol before adding Ligation Solution C.

5.2 Ligation of Linear DNA

Components	Volume
DNA mix *1	5-10 μ l
Ligation Solution B	Equal Volume as above
Ligation Solution A	Double Volume of above

Reaction: 16°C for 30 min*2

*1: Recommended volume input of DNA fragment is 0.01-0.1 pmol.

The molar ratio of input DNA fragment and Linker DNA is recommended to be 1:100 or above.

*2: For unsatisfactory results, prolong the reaction time to overnight.

Incubation at 70°C for 10 min could deactivate the T4 DNA Ligase.



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