Quick DNA Ligation Kit

Technical Manual No. TM0235 **Version 04022007**

I. DESCRIPTION

Quick DNA Ligation Kit is designed for efficient and quick ligation of cohesive-ended, blunt-ended, T-A cloning and linker ligation. 2× Quick Ligation Buffer containing ATP contribute to high performance of ligation. The ligation reaction need no inactivity and can be directly transformed.

II. Kit Components

- Quick Ligation Buffer 200 µl
- T4 DNA Ligase (GenScript, Cat. No. E00016, 4 U/µl) 25 µl

III. SHIPPING AND STORAGE

This product is shipped on blue ice. Store the product at -20°C.

IV. QUALITY CONTROL

Ligate 1 µg Lambda DNA/*Hin*d III Marker (GenScript, Cat. No. MM1212) in 50 µl reaction system at 22°C for 5 min and then run the agarose gel.

V. GENERAL PROTOCOL USING QUICK DNA LIGATION KIT

A. Ligation

1. Add your DNA insert and vector into one tube with a 3:1 molar ration, and then adjust volume to 5 µl.
2. Add 5 µl of 2× Quick Ligation Buffer and mix.
3. Add 1 µl of T4 DNA Ligase and mix thoroughly.
4. Centrifuge briefly and incubate at 22°C or 16°C as the recommended time.
5. Chill on ice (Do not heat inactive).

B. Transformation

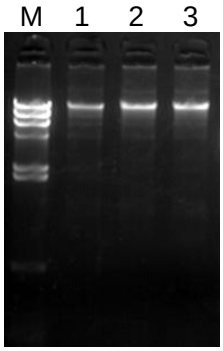
1. Briefly centrifuge the ligation reaction.
2. Add 2 µl of the ligation reaction to 50 µl competent cell.
3. Incubate on the ice for 30 min.
4. Heat-shock at 42°C for 2 min.
5. Chill on ice for 3 min.

6. Add 1 ml of LB to the tube, and then shake it at 200 rpm for 1 h.
7. Plate an appropriate amount of cells on the plates.
8. Incubate the plates at 37°C and grow overnight.

VI. APPLICATION EXAMPLES

1. Cohesive-end Ligation

Example 1: Lambda DNA/*Hin*d III Marker was ligated using the buffer for different times.



M: Lambda DNA/*Hin*d III Marker
Lane 1: Marker was ligated at 22°C for 5 min.
Lane 2: Marker was ligated at 22°C for 10 min.
Lane 3: Marker was ligated at 22°C for 15 min.

Fig. 1 Lambda DNA/*Hin*d III Marker Ligation

Example 2: 700 bp fragments from *Eco*R I and *Hin*d III double digestion were purified from an agarose gel. pUC57 vector (GenScript, Cat. No. SD1176) was also digested with *Eco*R I and *Hin*d III. The ligation reaction was incubated at R.T. for 5 min then transformed.

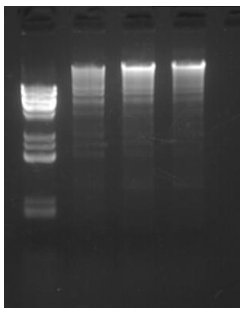
Vector	50 ng
Insert (3:1)	30 ng
dd H_2O	up to 5 µl
2× Quick Ligation Buffer	5 µl
T4 DNA Ligase (5 U/µl)	1 µl

Results (transformants/µg):

Control plasmid (uncut)	1.0×10^6
Vector without insert	5.5×10^3
Vector + insert	3.4×10^5

2. Blunt-end Ligation

Example 1: Lambda DNA/*Eco*R V Marker was ligated using the buffer for different times.



M: Lambda DNA/*Eco*R V Marker
Lane 1: Marker was ligated at 22°C for 5 min.
Lane 2: Marker was ligated at 22°C for 10 min.
Lane 3: Marker was ligated at 22°C for 15 min.

Fig. 2 Lambda DNA/*Eco*R V Marker ligation

Example 2: 1,000 bp fragments from *Eco*R V digestion were purified from an agarose gel. pUC57 vector was also digested with *Eco*R V and treated with Calf intestine Alkaline Phosphatase (CIAP) enzyme. The ligation reaction was incubated at R.T. for 15 min then transformed.

Vector	50 ng
Insert (3:1)	40 ng
dd H_2O	up to 5 µl
2× Quick Ligation Buffer	5 µl
T4 DNA Ligase (5 U/µl)	1 µl

Results (transformants/µg):

Control plasmid (uncut)	1.0×10^6
Vector without insert	0
Vector + insert	2.6×10^4

3. Plasmid Recircularization

Use the Quick Ligation Protocol with 50 ng of linearized vector without insert.
Example: pUC57 vector was cut with *Eco*R V, and then purified from an agarose gel. The ligation reaction was incubated at R.T. for 5 min then transformed.

Vector	50 ng
Insert	none
dd H_2O	up to 5 µl
2×Quick Ligation Buffer	5 µl
T4 DNA Ligase (5 U/µl)	1 µl

Results (transformants/µg):

Control plasmid (uncut)	8.2×10^5
Recircularized plasmid	1.4×10^6

4. T-A Cloning

Example: 500 bp fragments amplified with *Taq* DNA Polymerase (GenScript, Cat. No. E00007) was ligated with pUC 57-T vector. The ligation reaction was incubated at 16°C for 30 min then transformed.

Vector	50 ng
Insert (3:1)	50 ng
dd H_2O	up to 5 µl
2× Quick Ligation Buffer	5 µl
T4 DNA Ligase (5 U/µl)	1 µl

Results (transformants/µg):

Control plasmid (uncut)	1.0×10^6
Vector without insert	2.0×10^3
Vector + insert	1.8×10^4

VII. ORDER INFORMATION

Quick DNA Ligation Kit Cat. No.: L00244

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