A&A BIOTECHNOLOGY
innovating life science

Manual

Liga5™

5-minute ligation kit. For cohesive and blunt ends of DNA.

cat#	size
1108-25	25 reactions
1108-50	50 reactions
1108-150	150 reactions

For research use only.

Guarantee

A&A Biotechnology provides a guarantee on this product.
The company does not guarantee the correct performance of this kit in the event of:

- not adhering to the supplied protocol
- use of not recommended equipment or materials
- use of other reagents than recommended or which are not a component of the product
- use of expired or improperly stored product or its components

Advantages

- fast, 5-minute procedure
- up to 10 times higher yield compared to traditional ligation methods

Description

The Liga5™ kit contains a T4 phage DNA Ligase that catalyzes the formation of a phosphodiester bond between juxtaposed 5'-phosphate and 3'-hydroxyl termini in duplex DNA or RNA with blunt or cohesive ends. The enzyme repairs single stranded nicks in duplex DNA, RNA or DNA-RNA hybrids but has no activity on single stranded nucleic acids. The T4 phage ligase is purified from an *E. coli* bacterial strain carrying a plasmid with cloned gene *g30* of bacteriophage T4.

The Liga5™ kit improves on average 10 x ligation efficiency of blunt-ended nucleic acids and up to 3 x ligation of the cohesive ends, compared to traditional DNA ligation.
The Liga5™ ligation mix has no activity on single-stranded nucleic acids.

Contents

	1108-25	**1108-50**	**1108-150**	**storage**
Liga5™ enzyme	28 µl	55 µl	165 µl	-20 °C
Liga5™ buffer	140 µl	275 µl	825 µl	-20 °C
ultrapure water	1.5 ml	1.5 ml	2 x 1.5 ml	-20 °C – +20 °C

Notes

- Both cloning vector and insert DNA must be suspended in water or in Tris buffer (10 mM Tris HCl pH 8.0).
- It is recommended to use standard amounts of cloning vector and insert DNA in the ligation mixture. Molar ratio of the cloning vector to the insert DNA should be 1:3, (e.g. 0.020 pmol of the vector DNA to 0.060 pmol of the insert DNA).
- The ligase should not be heat inactivated. Heat inactivation significantly reduces transformation efficiency.
- It is not recommended to extend the ligation time over 10 min incubation.

Ligation protocol

1. Thaw and mix all component, place tubes on ice and add:

   Note. The quantities of vector and insert (ng) are an example for the vector of 4 kb and the insert of 1 kb.

	reaction volume
component	**20 µl**
Liga5™ enzyme	1 µl
Liga5™ buffer	5 µl
DNA vector	0.02 pmol*
DNA insert	0.06 pmol*
ultrapure water	up to 20 µl

   *DNA concentration calculation formula follow page 5

2. Mix by pipetting.
3. Incubate **5 min** at **room temp**. **(21°C - 23°C)**.
4. Keep on ice until transformation or store at **-20 °C**.

DNA concentration calculation formula

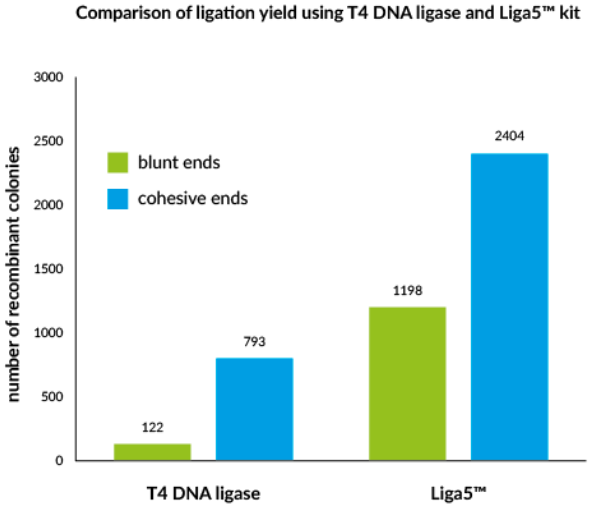
To calculate the number of pmoles of each fragment based on fragment length and weight, we recommend the following formula:

pmol = (weight in ng) x 1000 / (bp x 650 daltons)

length of DNA fragment (bp)	DNA fragment quantity (0.1 pmol)
200	6.5 ng
300	19.5 ng
500	32.5 ng
1000	65 ng
2000	130 ng
3000	195 ng
4000	260 ng
5000	325 ng

E. coli transformation

The reaction mix can be directly used to transform *E. coli* competent cells by either chemical methods or electroporation. For transformation we recommend E.coli Transformer Kit, cat. # 4020-240.



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