DNA Quick Ligation Kit

Catalog: RK20500

Size: 50 RXN / 200 RXN

Components:

Quick Ligase	RM21502
2X Quick Ligation Buffer	RM20107

Product Description

DNA Quick Ligation Kit equipped with efficient connection buffer and ligase. The Kit enables ligation of cohesive or blunt-end DNA fragments in 5 minutes at room temperature (25°C). It is applicable to cloning into vectors, library construction, T/A cloning, linker ligation, and recircularization of linear DNA.

Reaction Conditions: 1X Quick Ligation Buffer.

1X Quick Ligation Reaction Buffer:

66 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM DTT, 1 mM ATP, 7.5% Polyethylene glycol (PEG6000), pH 7.6 @ 25°C

Storage Temperature: -20℃

Instructions

Quick Ligation Protocol

1. Set up the following reaction in a microcentrifuge tube on ice. (*Quick Ligase should be added last. Note that the table shows a ligation using a molar ratio of 1:3 vector to insert for the indicated DNA sizes.*)

Composition	Amount
Nuclease-free dH_2O	up to a total volume of 20 µl
2X Quick Ligation Buffer*	10 µl
Vector DNA (4 kb)	50 ng (0.02 pmol)
Insert DNA (1 kb)**	37.5 ng (0.06 pmol)
Quick Ligase***	1 µl
Total Volume	20 µl

**2X Quick Ligation Buffer should be thawed and resuspended at room temperature.*

***Insert DNA (1 kb): a ligation using a molar ratio of 1:3 vector to insert for the indicated DNA sizes.*

****Quick Ligase should be added last.*

2. Gently mix the reaction by pipetting up and down and microfuge briefly.
3. Incubate at room temperature (25°C) for 5 minutes.
4. Chill on ice and transform 1-5 µl of the reaction into 50 µl competent cells. Alternatively, store at -20°C.
5. Do not heat inactivate. Heat inactivation dramatically reduces transformation efficiency.

Notes:

Some of the most critical parameters which should be controlled to ensure successful ligation and transformation are addressed below.

- Cells: Competent cells can vary by several logs in their competence. Perceived ligation efficiency directly correlates to the competence of the cells used for transformation. Always transform the uncut vector as a control for comparison purposes.
- Electroporation: Electroporation can increase transformation efficiency by several logs. Before using the products of a DNA Quick Ligation reaction for electrotransformation, it is necessary to reduce the PEG concentration. We recommend a spin column purification.
- DNA: Purified DNA for ligations can be dissolved in dH_2O (Milli-Q™ water or equivalent is preferable); TE or other dilute buffers also work well. For optimum ligation, the volume of DNA and insert should be 10 µl before adding 2X Quick Ligation Buffer. For DNA volumes greater than 10 µl, increase the volume of 2X Quick Ligation Buffer such that it remains 50% of the reaction

and correspondingly increase the volume of ligase. The overall concentration of vector + insert should be between 1-10 μg/ml for efficient ligation. Insert: vector ratios between 2 and 6 are optimal for single insertions. Ratios below 2:1 result in lower ligation efficiency. Ratios above 6:1 promote multiple inserts. If you are unsure of your DNA concentrations, perform multiple ligations with varying ratios.

- Time: Most ligations performed using the DNA Quick Ligation Kit reach an endpoint in 5 minutes or less at 25°C. Incubation beyond this time provides no additional benefit. In fact, transformation efficiency starts to decrease after 1 hour and is reduced by up to 75% if the reaction is allowed to go overnight at 25°C.
- Biology: Some DNA structures, including inverted and tandem repeats, are selected against by *E. coli.* Some recombinant proteins are not well tolerated by *E. coli* and can result in poor transformation or small colonies.