

Quick Ligase



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Quick Ligase

Quick Ligase is an ATP-dependent recombinant enzyme isolated from *Escherichia coli* strain and used to clone DNA in just 5 to 15 minutes.

Quick Ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5'-phosphate and 3'-hydroxyl termini in duplex DNA or RNA. It will join both blunt-ended and cohesive-ended restriction fragments of DNA, as well as repair single-stranded nicks in duplex DNA, RNA or DNA/RNA hybrids.



Applications

- Ligation in just 5–15 minutes
- Cloning PCR products
- Cloning restriction fragments
- Joining double-stranded oligonucleotide linkers or adaptors to DNA
- Site-directed mutagenesis
- Nick repair in duplex DNA, RNA or DNA/RNA hybrids
- Self-circularization of linear DNA
- LM PCR methods (Ligation Mediated PCR), e.g. amplified fragment length polymorphism (AFLP)

Quick Ligase

Protocol

1. Add the reaction reagents listed below to a sterile nuclease-free Eppendorf tube placed **on ice in a freezing rack**. The reaction agents should be added in the following order:

Component	Volume
Vector DNA	x μ l (20 – 50 ng)
Insert DNA	y μ l (3 – 10 molar excess over vector)*
2x Quick Ligation Buffer	10 μ l
ATP Solution (25 mM)	0.4 – 0.8 μ l
Quick Ligase (5 U/ μ l)	1 μ l
Nuclease-free water	up to 20 μ l

* A lower ratio will result in a less efficient ligation; a higher ratio will incite multiple insertions. This data is intended for use as a guide only; conditions will vary from reaction to reaction and may need optimization.

2. Mix gently and spin briefly.
3. For cohesive (sticky) ends, incubate at 20–25°C for 5 minutes. For blunt ends incubate at 20–25°C for 15 minutes.
4. Cool the samples on ice and transform 1–5 μ l of the reaction mixture into 50 μ l *E. coli* competent cells.



Additional information

- The 2x Quick Ligation Buffer and ATP Solution should be thawed and resuspended at room temperature.
- For blunt-end ligations, use higher quantities of both the vector and the insert DNA.
- For sticky (cohesive)-end ligations, we recommend heating both the vector and the insert DNA prior to ligation.
- The electrotransformation efficiency may be improved by heat inactivation of the **Quick Ligase** and purification of the DNA by means of a spin column purification method (**EXTRACTME DNA CLEAN-UP** kit or **EXTRACTME DNA GEL-OUT** kit).
- We recommend using a 3–10 molar excess of insert DNA over vector DNA. To calculate the optimal amounts of insert DNA in a ligation reaction, use the following equation:

$$\text{ng of insert} = \frac{\text{ng of vector} \times \text{kb size of insert} \times \text{molar ratio insert:vector}}{\text{kb size of vector}}$$

Example: If using 20 ng of a vector plasmid (4 kb), for a 5:1 molar ratio of insert:vector, you will require the following quantity of 1 kb insert:

$$\frac{20 \times 1 \times 5}{4} = 25 \text{ ng}$$

- The enzyme is inhibited by >200 mM NaCl or KCl concentrations.
- Inactivate enzyme at 65°C for 10 minutes or at 70°C for 5 minutes.

Storage buffer

10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 50 mM KCl, 50% (v/v) glycerol

2x Quick Ligation Buffer

132 mM Tris-HCl (pH 7.6), 20 mM DTT, 20 mM MgCl₂, 15% PEG 6000

The 2x Quick Ligation Buffer does not contain ATP, which must be added separately. For most applications, ATP should be added to the reaction to a final concentration of 0.5–1.0 mM. 25 mM ATP Solution is included.

Quality control

The product was tested in ligation of *Hind*III-cut lambda DNA with a different amounts of enzyme. The product is free of unspecific DNA nucleases.

Unit definition

One (Weiss) unit of **Quick Ligase** catalyzes the conversion of 1 nmol of ³²P from pyrophosphate into Norit-adsorbable material in 20 minutes at 37°C.

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Components	EN12-050 50 reactions	EN12-150 150 reactions	EN12-S 5 reactions
Quick Ligase 5 U/ μ l	50 μ l	3x 50 μ l	5 μ l
2x Quick Ligation Buffer	500 μ l	3x 500 μ l	50 μ l
ATP Solution (25 mM)	80 μ l	3x 80 μ l	8 μ l


Storage & shipping

Storage conditions

All components should be stored at -20°C . When stored under optimum conditions, the reagents are stable for at least 12 months from date of purchase.

Shipping conditions

Shipped on dry or blue ice.

 For research use only