

# USB® Ligate-IT™ Rapid Ligation Kit



## Quick ligation in just 5 minutes

- Ligation reactions complete in 5 minutes for cohesive ends and 10 minutes for blunt ends
- Convenient, ready-to-use format
- Room temperature incubation
- Flexible - useful in a variety of ligation procedures
- Ligation products can be directly used for transformation

## Quick and efficient

Ligation reactions are complete following room temperature incubation in just 5 minutes for cohesive-ends and 10 minutes for blunt-ends (see Figs. 1-2). Transform competent cells immediately or store at -20°C for later transformation.

## Convenient

The Ligate-IT Rapid Ligation Kit includes USB T4 DNA Ligase, Nuclease-Free Water, and a specially formulated 5X Reaction Buffer which enhances the activity of T4 DNA Ligase, especially on blunt-end fragments. All of the components are in a ready-to-use format and do not require any additional reagents, such as ATP.

## Flexible

The kit can be used for a variety of ligation procedures such as conventional vector cloning, TA cloning, linker or adaptor ligation, and library construction.

## Tested User Friendly™ functional test:

Cohesive- and blunt-end ligations of a 1200 bp insert into pUC19 yielded  $\geq 1 \times 10^6$  transformants per microgram of linearized, dephosphorylated vector.

## Ligate-IT Rapid Ligation Kit

Product code	Pack size
78400	25 reactions
78410	100 reactions

## Kit components

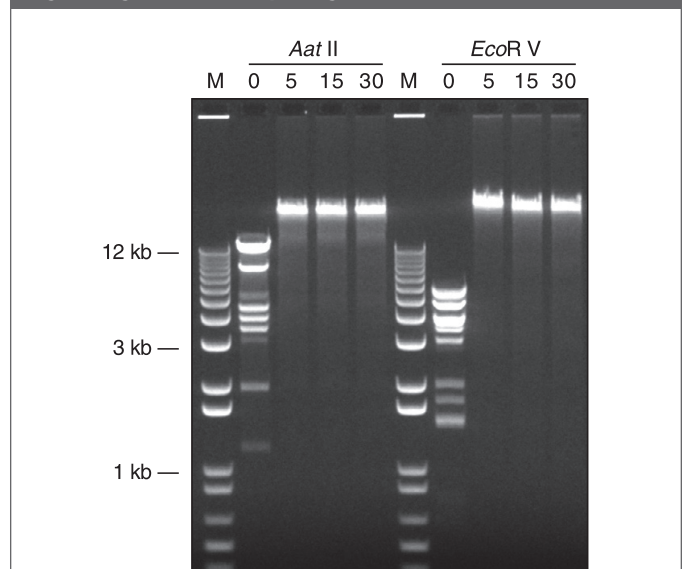
	25 reaction pack size	100 reaction pack size
Ligate-IT T4 DNA Ligase [PN 78401]	25 $\mu$ l	100 $\mu$ l
5X Ligate-IT Reaction Buffer [PN 78402]	100 $\mu$ l	400 $\mu$ l
Water, Nuclease-Free [PN 71786]	1 ml	2 x 1 ml

Recombinant T4 DNA Ligase is supplied in 25 mM Tris-HCl (pH 7.6), 100 mM NaCl, 1 mM DTT, 0.5 mM EDTA, 50% glycerol.

## Shipping and storage:

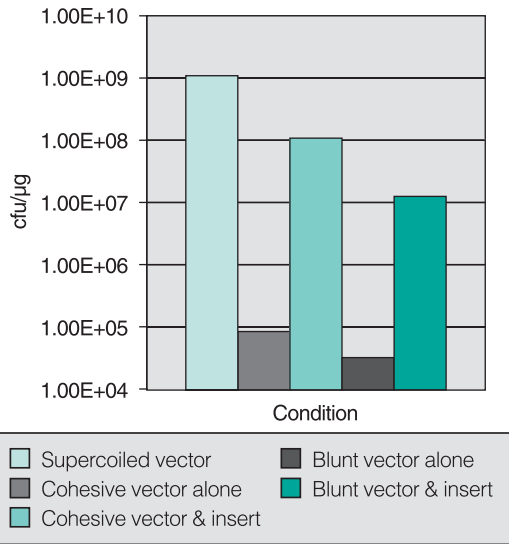
Kit shipped on dry ice. Store components at -20°C in a non-frost-free freezer.

Fig. 1. Ligate-IT Kit rapid ligation



Rapid ligation of cohesive-end and blunt-end fragments. Lambda DNA was cut with *Aat* II (cohesive-end fragments) or *EcoR* V (blunt-end fragments) and purified. In each lane, 500 ng of cut lambda DNA were ligated with the Ligate-IT Rapid Ligation Kit for the indicated times (in minutes) and then loaded onto a 1% agarose gel. M are the DNA marker lanes. Results demonstrate fast and efficient ligation of both cohesive-end and blunt-end fragments in just 5 minutes.

**Fig. 2. Ligase-IT Kit transformation efficiency**



Ligase-IT Rapid Ligation Kit transformation efficiency of cohesive-end and blunt-end ligations. A modified pUC19 vector was cut to generate cohesive-ends (*Aat* II and *Eco*O109 I) and blunt-ends (*Stu* I). Both vectors were dephosphorylated prior to ligation. The lac repressor (*lac*) from *E. coli* was cloned into the modified pUC19 vector with matching restriction sites for both cohesive-end and blunt-end ligations. Transformation efficiencies were determined by counting white colonies and are expressed in colony forming units per microgram of DNA used during transformation (cfu/µg). The uncut, supercoiled control vector was pUC19-lacI. Results indicate at least 10<sup>6</sup> transformants are obtained for both cohesive-end and blunt-end ligations when using competent cells that yield >10<sup>8</sup> cfu/µg.

**Protocol for ligation:**

Ligase-IT T4 DNA Ligase and 5X Ligase-IT Reaction Buffer have been functionally tested in the following protocol:

1. Thaw 5X Ligase-IT Reaction Buffer [PN 78402], mix thoroughly, and place on ice. Keep Ligase-IT T4 DNA Ligase [PN 78401] on ice at all times.
2. Mix 50-100 ng of vector DNA with a 1- to 3-fold molar excess of insert DNA. Bring volume to 15 µl with nuclease-free water [PN 71786]. (see Brief Protocol for molar ratio calculations).
3. Add 4 µl of 5X Ligase-IT Reaction Buffer and mix by pipetting.
4. Add 1 µl Ligase-IT T4 DNA Ligase, mix gently, and centrifuge briefly if necessary.
5. Incubate at room temperature (20-25°C) for 5 to 10 minutes. Cohesive and blunt end reactions are generally complete after about 5 and 10 minutes respectively. There are no benefits to increasing incubation time.
6. Following incubation, place on ice. Transform immediately or store at -20°C for later transformation. Generally, use 2-5 µl of ligation reaction per 50 µl competent cells. Follow manufacturer's instructions or standard protocols for transformation procedure<sup>(1)</sup>.

**References:**

1. Sambrook, J. and Russell, D. W. (2001) "Molecular Cloning: A Laboratory Manual", Cold Spring Harbor Laboratory Press, pp. 1.105-1.125.

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