

DNA Ligation Kit

 [cat info](#)

<Mighty Mix >

 [manual](#)

The DNA Ligation Kit <Mighty Mix > is a 2X premix, including T4 DNA Ligase and an optimized buffer system. It allows very rapid DNA ligation reactions. This kit improves our DNA Ligation Kit Ver.2.1 with even easier operation procedure: simply mix DNA solution with Ligation Mix and analyse (i.e. bacterial transformations or *in vitro* packaging). This kit achieves equivalent or higher efficiency in blunt-end ligation and TA cloning compared with the former versions. Owing to higher efficiency of the ligation reaction, conventional overnight incubations are no longer required. Good ligation efficiency is achieved in only five minutes for general ligation reactions, and most other types of ligations can be completed within 30 minutes.

Features

- **High Efficiency:** Facilitates highly-efficient ligations for cohesive, blunt-end, and TA cloning.
- **Convenient:** One solution premix eliminates pipetting steps, and reaction mixture can be directly used in transformations without purification.
- **Fast:** Reactions can be performed in either 30 min. at 16°C or 5 min. at 25°C, depending on DNA ends.
- **Small volumes:** Reaction in as few as 10µl.

Applications

Sticky-end ligation

564 bp fragment of λDNA, digested with *Hind* III (2.5-150 fmol), was added into pUC118 *Hind* III/BAP (Cat.#3324) (50 ng, 25 fmol). 7.5 µl of this DNA solution was added to 7.5 µl Ligation Mix <Mighty Mix> and incubated at 16°C for 30 min. After reaction, 10µl of the reactant was applied to *E. coli* JM109 competent cells (7.3×10^8 cfu/µg pUC118 DNA) for transformation. Transformed bacteria were plated on L-amp plate containing X-Gal and IPTG to form colonies.

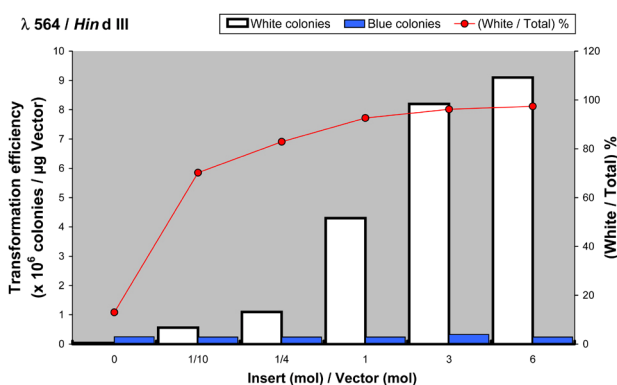


Fig.1 Sticky-end ligation efficiency with <Mighty Mix>

Blunt-end Ligation

500 bp fragment of λDNA, digested with *Hinc* II (2.5-150 fmol), was added into pUC118 *Hinc* II/BAP (Cat.#3322) (50 ng, 25 fmol). 7.5 µl of this DNA solution was added to 7.5 µl Ligation Mix <Mighty Mix> and incubated at 16°C for 30 min. After reaction, 10µl of the reactant was applied to *E. coli* JM109 competent cells (7.3×10^8 cfu/µg pUC118 DNA) for transformation. Transformed bacteria were plated on L-amp plate containing X-Gal and IPTG to form colonies.

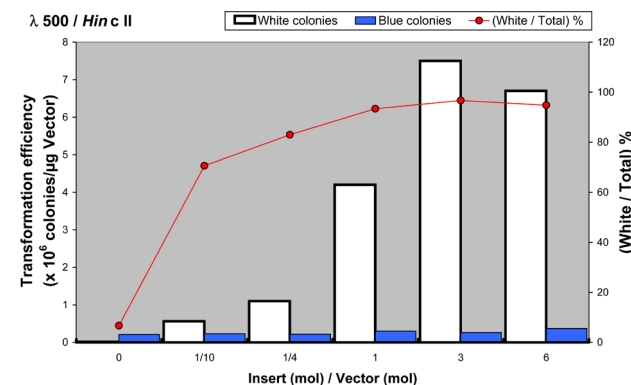


Fig.2 Blunt-end ligation efficiency with <Mighty Mix>

Competitor comparison of ligation efficiency with TA-cloning

Ligation efficiencies compared between Takara DNA Ligation Kit <Mighty Mix> and four competitors demonstrated that Takara's Mighty Mix produced more transformants (1.3×10^6 colonies/µg vector) than the highest efficiency competitor kit (7.3×10^5 colonies/µg vector). Figure below shows TA cloning efficiency of the Mighty Mix versus several competitors.

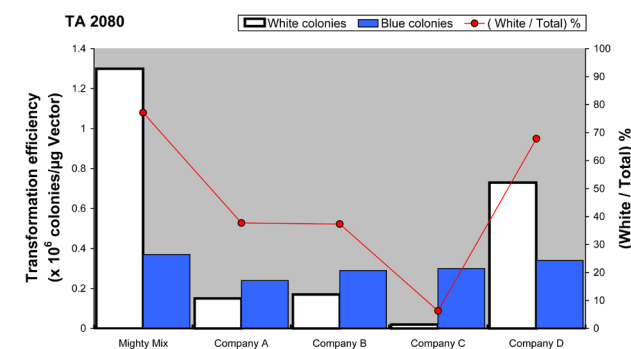


Fig.3 TA-Vector ligation efficiency comparison of Takara's DNA Ligation kit <Mighty Mix> against four competitors.

In addition, comparison of Takara Mighty Mix ligation efficiencies using the 5 min. vs. 30 min. protocols demonstrates better efficiency using the 5 min. & 25°C protocol (9.5×10^6 colonies/µg vector) with sticky ends. For blunt-end ligation better efficiency was obtained with 30 min. & 16°C protocol.

In both cases efficiency of Mighty Mix outperforms the highest competitor kit efficiency.