

FAQs

DNA Ligation Kits

1. What are the differences between all of Takara's DNA Ligation Kits?

Takara has five ligation products to suit any DNA ligation need:

DNA Ligation Kit, Mighty Mix - a new single premix solution that offers quick, high efficiency ligation reactions, even for blunt-ended and TA-cloning reactions, in 5 minutes at 25°C.

DNA Ligation Kit, Version 2.1, - our most popular ligation kit that provides simple ligation reactions for circular sticky-ended plasmids in 5 minutes at 25°C. The Kit uses a single ligation solution, which allows low volume ligations in instances where DNA amounts may be limiting. Furthermore, transformation efficiency can be improved by addition of the Transformation Enhancer solution to the ligation reaction mixture before transformation into competent cells.

DNA Ligation Kit, Version 1.0. - recommended for linear ligations, such as λ DNA concatenations, as well as circular plasmid ligations, this kit provides nearly equivalent transformation efficiencies as does the Version 2.1 Kit. The kit is composed of two ligation solutions, rather than a single solution.

DNA Blunting Kit - this kit allows blunting of DNA fragments with dephosphorylated 5'-protruding ends, but not those with phosphorylated 3'-recessed ends, which are often present in sonicated DNA. The same two ligation solutions that are provided with the DNA Ligation Kit, Version 1.0 are also provided with this product.

BKL Reagent Set - this kit allows blunting of PCR products which are to be cloned into blunt-ended vectors, with 5'-end phosphorylation. The same single ligation solution that is provided with the DNA Ligation Kit, Version 2.1 is also provided with this product.

2. How can I stop a ligation reaction:

DNA ligase can be denatured by heating the ligation mixture to 70°C for 7 minutes or by EtOH precipitation.

3. In general, how can I improve transformation efficiencies using my ligated DNA?

If it is necessary to improve transformation efficiencies, then we suggest trying the following recommendations :

- a) Extend the ligation reaction time to overnight at 16°C, to improve the number of ligated molecules.
- b) Add NaCl to a final conc. 500 mM into the ligation mixture prior to transformation.

EtOH precipitation is recommended for clean up of the DNA if a large volume of ligation mixture is needed for transformation, or when performing electroporation.

4. Is it possible to ligate two linear pieces of DNA together?

Yes, it is possible to ligate two linear pieces of DNA together. We recommend, however, that you stay below the 5 kb size range. The efficiency of ligation depends upon size. Re-circularization of the pieces can be prevented by dephosphorylation of the non-joining ends.

5. What are the recommended conditions for ligation of a large circular plasmid with a comparatively small DNA insert?

Using a large circular vector (e.g. 20 kb) which has cohesive ends, and a small DNA insert (e.g. 1-2 kb), we recommend that you:

- a) Use at least 100 ng of vector.
- b) Use a mole ratio of vector:insert = 1:3-5.
- c) With Kit Version 2.1, for increasing the effective termini of DNA, heat the DNA solution containing vector and insert at 65°C for 2 minutes and subsequently cool it on ice. Then, add the Solution I into this DNA solution and incubate at 16°C for 0.5-3 hours.
- d) For transformation, use an *E.coli* strain, such as DH5 alpha, that is capable of receiving large plasmids.

6. I note that the Version 1.0 Kit is recommended for blunt-ended ligations. How do I improve my ligation efficiencies when performing a blunt-end ligation reaction?

The larger the vector, the more difficult the ligation, therefore try to maintain a vector size that is only 20-50% larger than the insert.

To improve the efficiency of blunt-end ligations, specifically when using the Version 1.0 Kit, please follow the suggestions below:

- a) The use of BAP (bacterial alkaline phosphatase), vs. CIAP, is recommended for dephosphorylation of the vector. Dephosphorylation with CIAP may be insufficient.
- b) If a gel-purified insert DNA is used for ligation, then DNA clean-up by EtOH precipitation is recommended prior to ligation.
- c) Use 100 ng (rather than 50 ng) of the vector for ligation, if possible.
- d) Recommended molar ratio is vector:insert = 1:5-10.
- e) Add 8X of Solution A of the Version 1.0 Kit and mix thoroughly. Then add 1X of Solution B and mix thoroughly.
- f) Incubate at 16°C for several hours or overnight. Incubation at room temperature may inhibit the circularization of DNA

7. How do I clean up ligated DNA in order to digest it with restriction enzymes?

To perform a restriction enzyme digestion using the ligated DNA, we strongly recommend cleaning the ligated DNA via EtOH precipitation, in order to avoid inhibition of the digestion reaction by the ligation solution.

8. Can the ligation reaction mixture be used directly for PCR?

Because of the Mg²⁺ concentration of the Ligation Solutions in our kits, we do not recommend that the ligation reaction be used directly for PCR. Instead, clean-up the DNA by EtOH precipitation prior to PCR.