DNA Ligation Kit, Mighty Mix (Cat.# 6023)

Protocol-at-a-Glance: DNA Ligation Kit, Mighty Mix

Takara's DNA Ligation Kit, Mighty Mix (Cat. # 6023), is a premixed ligation solution that enables highly efficient ligation. It can be used for many types of applications including standard cloning (sticky and blunt end), T-vector cloning, self-circularization, linker ligation, and preparation of λ phage library.

The following Protocols-At-A-Glance provide brief instructions for ligations commonly performed with this product. For more information, refer to the product User Manual.

Protocol 1: Insertion of DNA fragments into plasmid vectors

The Standard Protocol should be used for general ligation reactions. When performing sticky-end DNA ligations or when the highest efficiency is not required, the Rapid Protocol offers good efficiency in a shorter period of time.

[Standard Protocol]

1. Combine the digested plasmid vector DNA and the DNA insert in a total volume of 5 - 10 µl. We recommend using TE buffer (10 mM Tris- HCl, pH 8.0, 1 mM EDTA) for dissolving DNA. The recommended vector : insert ratio is 25 fmol vector : 25 - 250 fmol insert. (Note : 25 fmol of pUC118 DNA (3,162 bp) corresponds to approx. 50 ng).
2. Add one volume of Ligation Mix (5 - 10 µl) to the DNA solution and mix thoroughly.
3. Incubate at 16˚C for 30 minutes*1.
4. The ligation reaction mixture can be used directly for transformation of *E. coli* competent cells. When performing transformation immediately following ligation, add 10 µl of the ligation mixture to 100 µl of competent cells*2.

*1: This incubation may be performed overnight.

*2: If more than 10 µl of the ligation reaction mixture must be used for transformation, then the ligated DNA should be ethanol precipitated prior to use.

NOTE: The ligation reaction mixture should not be used directly in electroporation. Instead, ligated DNA should be ethanol precipitated and dissolved in a low salt buffer, such as TE buffer, prior to use for electroporation.

[Rapid Protocol]

1. Combine the digested plasmid vector and the DNA insert in a total volume of 5 - 10 µl. We recommend using TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) for dissolving DNA. Recommended vector : insert ratio is 25 fmol vector : 25 - 250 fmol insert (Note : 25 fmol of pUC118 DNA (3,162 bp) corresponds to approx. 50 ng).
2. Add one volume of Ligation Mix (5 - 10 µl) to the DNA solution and mix thoroughly.
3. Incubate at 25°C for 5 minutes*1.
4. The ligation reaction mixture can be used directly for transformation of *E.coli* competent cells. When performing transformation immediately after ligation, add 10 µl of the ligation mixture to 100 µl of competent cells*2.

*1: Higher temperatures (>26°C) will inhibit the formation of circular DNA. When performing the Rapid Protocol, maintain the reaction temperature at 25°C.

*2: If more than 10 µl of the ligation reaction mixture must be used for transformation, then the ligated DNA should be ethanol precipitated prior to use.

NOTE: The ligation reaction mixture should not be used directly in electro¬poration. Instead, ligated DNA should be ethanol precipitated and dissolved in a low salt buffer, such as TE buffer, prior to use for electroporation.

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Protocol 2: Cloning a PCR product into a T-Vector

1. Prepare a DNA solution*1 containing 13 - 25 fmol of T-Vector (e.g. approximately 25 - 50 ng of pT7 Blue T-Vector (Novagen)) and 5 - 250 fmol insert DNA in a total volume of 5 - 10 µl.
2. Add one volume (5 - 10 µl) of Ligation Mix into the DNA solution and mix well by pipetting.
3. Incubate at 16°C for 30 minutes*2.
4. The ligation reaction mixture can be used directly for transformation of *E. coli* competent cells. When performing transformation immediately after ligation, add 10 µl of the ligation mixture to 100 µl of competent cells*3.

*1: We recommend dissolving the DNA in TE buffer (10 mM Tris-HCl,1 mM EDTA, pH 8.0).

*2: When ligating PCR products into T-Vector, the reaction should be complete within 1 hour. Longer reaction times may result in higher background.

*3: If more than 10 µl of the ligation reaction mixture must be used for transformation, then the ligated DNA should be ethanol precipitated prior to use.

Protocol 3: Self-circularization of linear DNA (Intramolecular ligation)

The procedure for self-circularization of linear DNA is essentially the same as for Protocol 1. Insertion of DNA fragments into plasmid vectors. However, it is important to use low concentrations of DNA in the ligation reaction to maximize intramolecular ligation and to keep the volume of the DNA solution low for higher transformation efficiency.

Protocol 4: Linker [Adaptor] ligation

1. Linker ligation to a plasmid vector

The procedure for linker ligation are essentially the same as for Protocol 1. Insertion of DNA fragments into plasmid vectors. The recommended vector/linker molar ratios are :

- phosphorylated linker : dephosphorylated vector = 10 - 1,000 : 1
- phosphorylated linker : phosphorylated vector = greater than 100 : 1

2. Linker ligation to termini of a DNA fragment

1. Prepare 5 - 10 µl of DNA solution containing DNA fragment to be ligated (10 - 100 fmol) and linker (or adaptor). The recommended DNA fragment : linker[adaptor] molar ratio is :

- DNA fragment : linker [adaptor] = 1 : greater than 100

2. Add Ligation Mix in an amount that is twice the volume (10 - 20 µl) than that of the DNA solution, and incubate at 16°C for 30 min. However, if the linker is shorter than 8 bases or if the linker has a low GC-content, perform the ligation reaction at a lower temperature (<10°C) for 1 to 2 hours.

3. If the ligated DNA is to be further subjected to restriction enzyme digestion, then ethanol precipitate and resuspend the DNA in an appropriate buffer prior to digestion.

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Protocol 5: Insertion of DNA fragments into a λ phage vector

1. Prepare a DNA solution containing 250 ng of λ phage vector (10 fmol) and DNA fragment (30 - 100 fmol) in a total volume of 2.5 - 5 µl.

2. Add twice the volume of Ligation Mix (5 - 10 µl) to the DNA solution and mix well.

3. Incubate at 26°C for 5 - 10 minutes. A higher efficiency is obtained at 26°C than at 16° C. Longer reaction times may lower the efficiency. Therefore, limit the reaction time to 5 - 10 minutes.

4. Perform in vitro packaging.

The ligation reaction mixture can be used directly for packaging. The composition of Ligation Mix will not inhibit the λ packaging reaction as long as the ratio of ligation reaction mixture to the total packaging reaction mixture is kept to <10% of the packaging lysate, even when using a commercially available packaging kit (e.g. MaxPlax Lambda Packaging Extracts (EPICENTRE Technologies), GigaPack (Stratagene)). Generally, 4 µl of ligation reaction mixture can be added per packaging reaction. If the total amount of the ligation reaction mixture must be added to a packaging reaction, then the DNA should first be ethanol precipitated and redissolved in TE buffer in order to bring the DNA volume to <10% of the packaging lysate.