PRODUCT INFORMATION

Product Name: DynaExpress DNA Ligation Kit ver.2
Code No: DS110
Size: 50-200 reactions *This product is research use only*

Description:

This DNA Ligation Kit enables ligation of cohesive end or blunt end DNA fragments within 5-30 minutes at 16 ℃-25 ℃. The DNA Ligation Kit is designed for fast and efficient ligation of both cohesive end and blunt ends. All necessary reagents required for ligation are provided. Simply ligation reaction can be started by adding 2x Ligation Buffer and Ligase Mixture to a mixture of vector and insert DNA solution. The ligation reaction mixture can be used directly for the transformation of chemical competent cells.

Application:

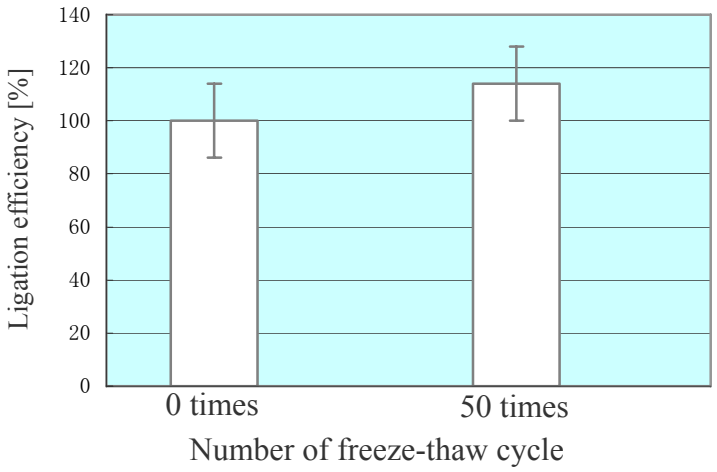
- Cloning in plasmid vectors
- TA cloning
- Recirculation of linear DNA

Kit Components:

2x Ligation Buffer	500 µl x 4 tubes
Ligase Mixture	50 µl

Storage: Store at -20 ℃

The 2x Ligation Buffer can be thawed and frozen repeatedly as shown in a figure below.



Contaminants Activity:

DNase: When 0.4 µg of λ/*Hin*d III digest dephospholyrated by alkaline phosphatase was incubated with a mixture of 10 µl of the 2x Ligation Buffer and 1µl of the Ligase Mixture for 16 hours at 37 ℃, no degradation of the DNA fragment was observed on agarose gel electrophoresis.

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RNase: No RNase activity was detected by the use of RNaseAlert assay (Ambion). In this assay the reaction mixture containing a fluorescent-labeled RNA substrate was incubated with a mixture of 10 µl of the 2x Ligation Buffer and 1µl of the Ligase Mixture for 1 hour at 37 ℃.

Quality Control: Each lot of the DNA Ligation Kit is pre-tested in a ligation reaction and transformation efficiency according to the following method.

The pBluescript SK(+) vector (Stratagene) is digested with *Eco*RV, dephosphorylated with alkaline phosphatase (PAP code No. DE110) and gel purified with gel indicator (code No. DM510). A blunt-ended 1kb DNA fragment is ligated to the vector at a 3:1 insert:vector ratio using the DNA Ligation Kit. Competent Cell DH5α (code No. DE220) are directly transformed with the ligation product and plated on X-gal / IPTG / Amp plates. Yield of white colonies of each lot is more than 10^6 per µg DNA.

Ligation Protocol:

1. Mix vector DNA and insert DNA to a tube and adjust volume to total 10 µl by dH_2O. *1
2. Add 10 µl 2x Ligation Buffer. *2
3. Add Ligase Mixture. *3, *4
4. Incubate for 30 min at 16 ℃. *5
5. Use 2-5 µl of the ligation reaction mixture for transformation. *6, *7

*1 We recommend the use of 50-100 ng of vector DNA and a 3-fold molar excess of insert DNA to vector DNA per ligation. The amount of vector DNA should not exceed 200 ng in 20 µl of ligation reaction mixture.

*2 It is necessary to mix thoroughly the contents of the 2x Ligation Buffer prior to use.

*3 Amount of Ligase Mixture

Amount of Ligase Mixture should be modified depending on the kind of ligation reaction. The following amounts are recommended:

	Amount of Ligase Mixture
Blunt end ligation	1 µl
Cohesive end ligation	0.5 µl or 0.25 µl
TA cloning	1 µl
Linker ligation	1 µl

Half or quarter of the amount of Ligase Mixture (0.5 µl or 0.25 µl) is also sufficient for routine ligation experiment of blunt end (shown in figure of Example).

*4 It is possible to make and use the convenient mixture of 10 µl 2x Ligation Buffer and 1 µl Ligase Mixture without decrease in activity for a few weeks at -30 ℃.

*5 Five minutes incubation is sufficient for cohesive end ligation even if 0.25 µl of Ligase Mixture is used in the protocal.

*6 The ligation reaction mixture can be used directly for the transformation of chemical competent cells. However, it is necessary to use the volume of the ligation reaction mixture to no more than 10 % of the volume of competent cell. Heat inactivation of the ligation reaction mixture before transformation

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decreases transformation efficiency. The ligation reaction mixture can be stored at -20 ℃ until use for transformation without heat inactivation.

In the case of electro-transformation, we recommend a spin column purification or chloroform extraction followed by ethanol precipitation.

*7 The ligation reaction mixture can be analyzed by agarose gel electrophoresis by heat preparation at 65 ℃ for 10 minuets.

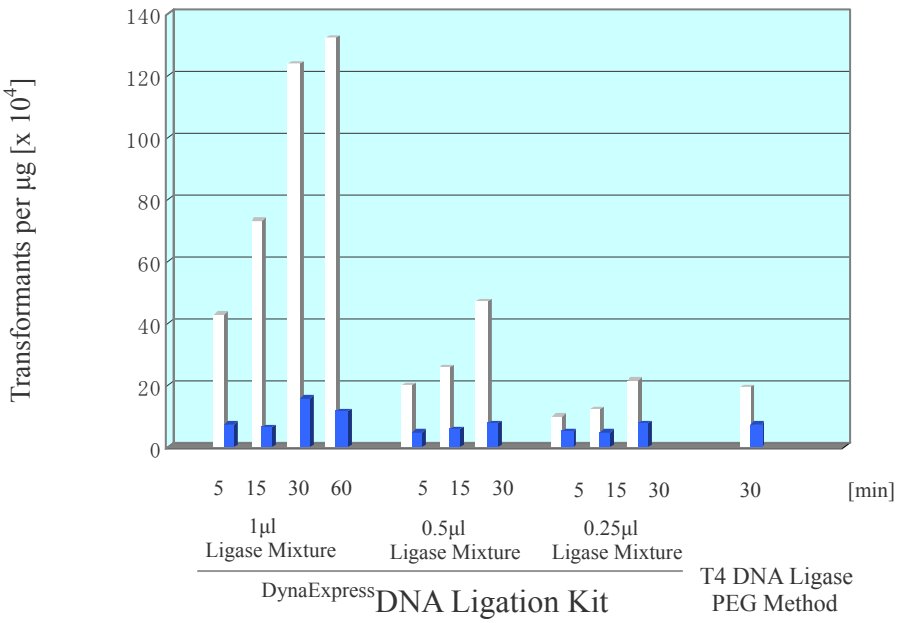
Transformation Protocol:

1. Transfer 2-5 µl of the ligation reaction mixture to a tube.
2. Add 50 µl of chemical competent cell to the ligation reaction mixture.
3. Incubate on ice for 30 min.
4. Heat shock for 45 sec at 42 ℃.
5. Add 450 µl of SOC medium.
6. Incubate at 37 ℃ for 1 hour.
7. Spread 100 µl on a plate.
8. Incubate overnight at 37 ℃.

Example:

A blunt-ended 1 kb DNA fragment was ligated to the *Eco*RV cleaved, dephosphorylated pBluescript SK(+) according to the standard protocol using the Ligation Kit.

*Eco*RV cleaved, dephosphorylated pBluescript SK(+)	2 µl	(100 ng)
Insert DNA (1 kb)	2 µl	(100 ng)
dH_2O	6 µl	
2x Ligation Buffer	10 µl	
Ligase Mixture	1, 0.5 or 0.25 µl	



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Troubleshooting:

Problem	Possible Cause	Solution
Few transformant	Incompatible ends	Check the ends of the DNA molecules.
	DNA contains inhibitors of ligation.	Dilute the DNA solution or purify the DNA by use of phenol extraction, agarose gel electrophoresis or column method.
	High salt concentration	Dilute the DNA solution or exchange the buffer by a spin column or ethanol precipitation.
	DNA has been damaged by UV.	Minimize UV exposure by use of Gel Indicator (code No. DM510).
	Cloned insert is not tolerated by *E.coli.*	Incubate the plate not at 37 ℃ but at room temperature. Use low copy number plasmid or promoter with a low expression background.
	The ligation reaction mixture was heat inactivated.	Don't heat inactivate. Store at -20 ℃ until use for transformation.
	Low competency of competent cells	Verify that the competent cells are active.
	Excess volume of the ligation reaction mixture was added to competent cell.	Use the volume of the ligation reaction mixture to no more than 10 % that of chemical competent cell.
	Incorrect vector: insert ratio	Check the amount of DNAs by electrophoresis or spectrophotometory and use correct ratio.
	Incorrect antibiotic	Check selective growth medium.
High background	Inactivation of antibiotic	Use the fresh selective growth medium.
	Incomplete digestion of vector DNA	Check linearization of vector DNA by gel electrophoresis. It is known to be difficult to completely digest vector DNA whose length is less than 4 kb. We recommend to purify the linearized vector DNA from agarose gel after electrophoresis by using Gel Indicator (Code No. DM510).
	Self recirculaization of vector DNA	Remove phosphate group from vector DNA by alkaline phosphatase to prevent recirculaization. We recommend alkaline phosphatase from a psychrotrophic bacterium *Shewanella sp.* SIB1 (PAP Code No.DE110).

Related Products:.

DM510	Gel Indicator Kit	DM550	Gel Indicator DNA Extraction Kit
DS220	Competent Cell DH5α	DS210	Competent Cell JM109
DS225	Jet Competent Cell (DH5α)	DM110	Alkaline phosphatase from *Shewanella sp.* SIB1

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