07-10

【High-Efficient Ligation Reagent】

Ligation high Ver.2

(Code No. LGK-201)

Instruction Manual

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A3706K

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●Caution

This product is for research use only. Never use this product for diagnostic purposes or for other clinical purposes. The potential hazards that may be caused by any component of this product have not been fully investigated. Adequate care should be taken when handling this product, e.g., use protective equipment and clothing.

[1] Introduction

The ligation reaction is an essential step in genetic recombination experiments. For this reaction, T4 DNA ligase has been widely used. Ligation high Ver.2 is a highly efficient premixed T4 Ligase reagent.

Features of Ligation high Ver.2

- Highly efficient*
  Effective ligation of cohesive, blunt and A overhang DNA fragments can be achieved.

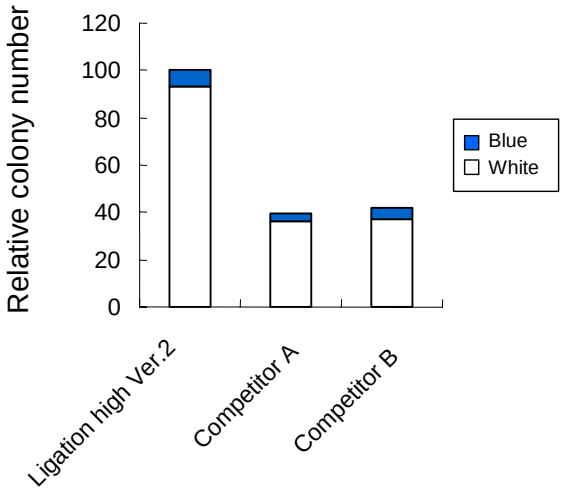

Fig. 1 Comparison of TA cloning efficiency
Colony number of Ligation high Ver.2 corresponds to 100

- Will not freeze at -20℃*
  Just use. No need to thaw.

- Ready to use
  Just mix ligation high Ver.2 with an equal volume or with double the volume of the solution containing DNA fragments.

*Patent pending

[2] Contents

●Contents
Ligation high Ver.2　　750 μl × 1Vial (100 rxn)

●Storage conditions
-20℃

※In the case of -30℃ storage or long-term storage under -20℃, the reagent might freeze. No decrease in reaction efficiency is observed following 5 freeze-thaw cycles.

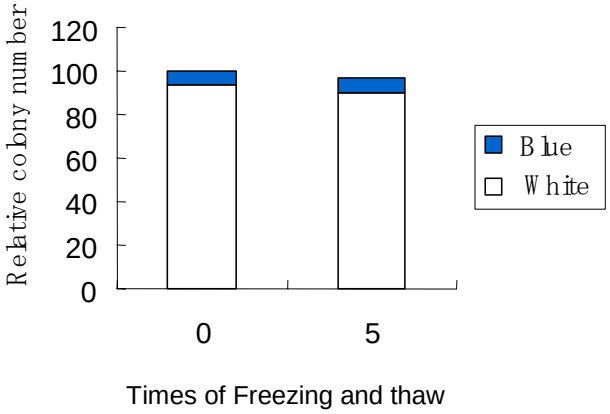

Fig. 2 Effect of freezing and thaw for activity of Ligation high Ver.2
Ligation efficiency was estimated by the TA cloning test.
Colony number at 0 times of freezing and thaw corresponds to 100
No negative effect was not detected by 5-time-freezing and thaw.

[3] Protocol

Vector DNA＋Insert DNA[*1]	7.5 μl
Ligation high Ver.2	3.75～7.5 μl[*2]
16℃、30 min.[*3*4]	
↓	
Transform 100 μl competent cells using ～10 μl reaction solution[*5]	

*1 Ligation efficiency is decreased by adding excess salts. DNA fragments should be in low salt solutions (e.g. 10 mM Tris-HCl[pH 8.0], 1 mM EDTA)

*2 Adding an equal volume of Ligation high Ver.2 to the DNA solution is recommended in the case of TA cloning.

*3 The reaction can be prolonged for up to 2 hr. Standard cohesive end ligations can be completed within 5 min.

*4 The reaction can be performed at 4℃～25℃.

*5 The volume of ligation reaction added should be less than 10% of the volume of the competent cell suspension. For electro-transformation, ligation reactions should be purified, because salts inhibit the transformation.

Table 1 Recommended reaction conditions

	DNA : Ligation reagent (Volume)	Reaction condition
Cohesive end ligation	1 : 1 or 1 : 0.5	16℃、5～30 min
Blunt end ligation	1 : 1 or 1 : 0.5	16℃、30 min
Linker ligation	1 : 1 or 1 : 0.5	16℃、30 min
T vector ligation (Purified PCR procuct)	1 : 1	16℃、30 min
T vector ligation [*1*2] (Unpurified PCR product)	1 : 1	16℃、30 min
Phage vector ligation	1 : 1 or 1 : 0.5	16℃、30 min

*1 When PCR reactions contain primer dimers or unexpected PCR products, the desired products should be purified.

*2 When sufficient amplification is confirmed, 0.5～1 μl of PCR product can be used directly. The reaction conditions are as follows;

H_2O	X
PCR product	0.5～1
Ligation high Ver.2	7.5
	15 (μl)

[4] Application data

(1) Cohesive end ligation

Dephosphorylated pUC19/*Hin*dIII,(50 ng, 25 fmol,)was mixed, at different ratios, with the 546 bp DNA fragment of *Hind*III digested phage lambda DNA. An equal volume of Ligation high Ver.2, 7.5 μl, was added and incubated at 16℃ for 30 min. *E. coli* DH5α competent cells were transformed using 10 μl of reaction mixture, and cultured on LB/Amp(X-Gal, IPTG) plates O/N.

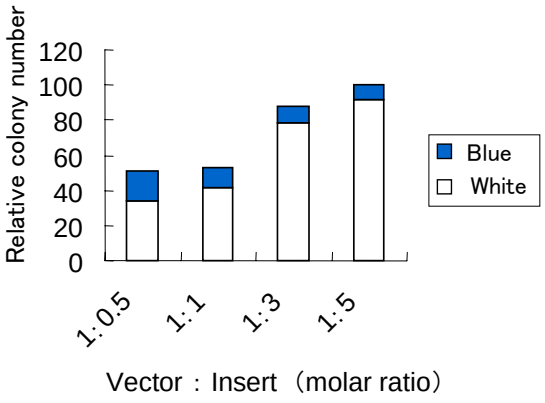

Fig. 3 Cohesive end ligation
Colony number at 1:5 corresponds to 100

(2) Blunt end ligation

Dephosphorylated pUC19/*Hin*cII,(50 ng, 25 fmol,) was mixed, at different ratios, with purified PCR product (500 bp: from high-fidelity PCR enzyme[*1]). Half or an equal volume of Ligation high Ver.2, 3.75 or 7.5 μl, was added and incubated at 16℃ for 30 min. *E. coli* DH5α competent cells were transformed using 10 μl of reaction mixture, and cultured on LB/Amp(X-Gal, IPTG) plates O/N.

[*1]High-fidelity PCR enzyme generates blunt ends.

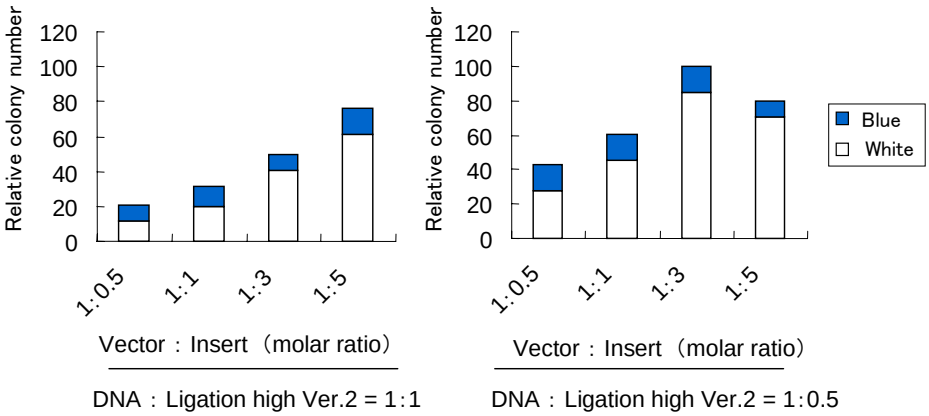

Fig. 4 Blunt end ligation
Colony number on the condition of DNA：Ligation high Ver.2 = 1:0.5 and Vector：Insert = 1:3 corresponds to 100.

(3) Linker ligation

Dephosphorylated pUC19/*Hin*cII,（50 ng, 25 fmol, was mixed at different ratios with ）phosphorylated *Hin*dIII linker (10mer). Half or an equal volume of Ligation high Ver.2, 3.75 or 7.5 μl, was added and incubated at 16℃ for 30 min. *E. coli* DH5α competent cells were transformed using 10 μl of reaction mixture, and cultured on LB/Amp(X-Gal, IPTG) plates O/N.

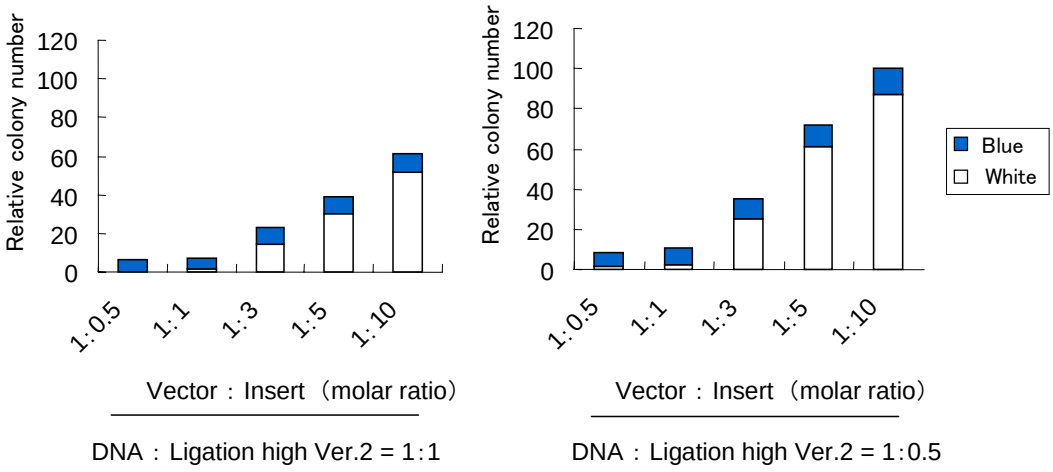

Fig. 5 Linker ligation
Colony number on the condition of DNA：Ligation high Ver.2 = 1:0.5 and Vector：Insert = 1:10 corresponds to 100.

(4) TA cloning using purified PCR prodcuts

T vector,（50 ng, 25 fmol,）was mixed, at different ratios, with purified PCR products (500 bp from Taq DNA polymerase). An equal volume of Ligation high Ver.2, 7.5 μl, was added and incubated at 16℃ for 30min. *E. coli* DH5α competent cells were transformed using 10 μl of reaction mixture, and cultured on LB/Amp(X-Gal, IPTG) plates O/N.

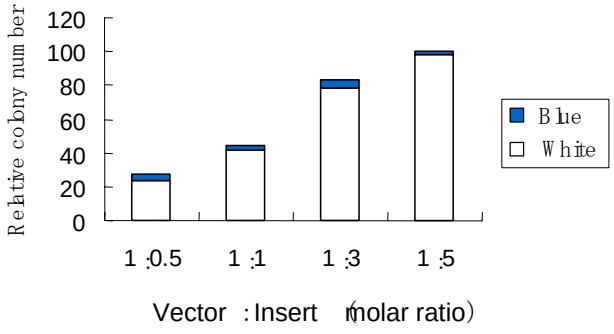

Fig. 6 TA cloning using purified products
Colony number at 1:5 corresponds to 100

(5) TA Cloning using unpurified PCR products

T vector,（50 ng, 25 fmol,） was mixed, at different ratios, with unpurified PCR products (500 bp from Taq DNA polymerase). An equal volume of Ligation high Ver.2, 7.5 μl, was added and incubated at 16℃ for 30 min. *E. coli* DH5α competent cells were transformed using 10 μl of reaction mixture, and cultured on LB/Amp(X-Gal, IPTG) plates O/N.

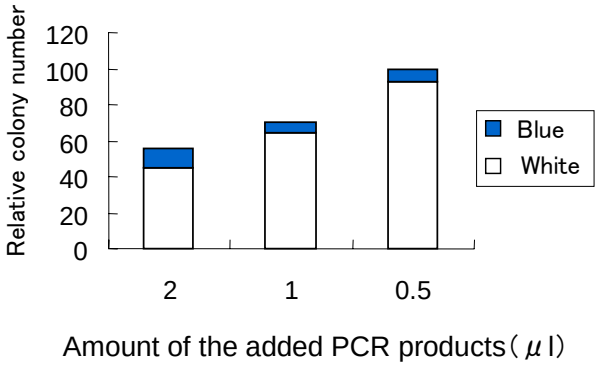

Fig. 7 TA cloning using unpurified products
Colony number on the condition of adding 0.5 μl of the PCR product corresponds to 100.

(6) Phage vector ligation

λZAP®II (500 ng), *Eco*RI digested, and pRheo/*Eco*RI test insert（225 ng, 2.8 kb）, were mixed with half or with an equal volume of Ligation high Ver.2, 3.75 or 7.5 μl, and incubated at 16℃ for 30 min. *in vitro* packaging was performed with 1.5 μl ligation reaction using GIGAPACK®III（Stratagene,） followed by infection of *E. coli* XL-1 Blue MRF'. The *E. coli* cells were cultured O/N and plaques counted.

Table 2. Reaction conditions and efficiency

DNA soln: Ligation reagent	1 : 0.5	1 : 1
Efficiency (pfu/μg ZAP®II)	6.3×10^6	5.5×10^6

[5] Trouble shooting

Trouble	Cause	Counter measures
No or low colony number	Excess amount of ligation mixture for transformation	Unpurified ligation mixtures should be added at less than 10%(V/V) of competent cell suspensions.
	Vector concentration is low	Increase the vector concentration. (→See p4～6)
	DNA insert fragment concentration is too low.	Increase the DNA insert fragment concentration. (→See p4～6)
	Excess salts	・ DNA fragments should be disolved in a low salt solution. ・ Unpurified PCR products should be added at 0.5～1 μl per ligation reaction.
	Ends of both vector and insert are dephosphorylated.	Dephosphorylated vectors cannot ligate with unphosphorylated PCR products.
Cloning efficiency was low	Primer dimer	When the PCR reaction contains primer dimers or unexpected PCR products, the desired PCR product should be purified.
	Insufficient time for ligation reaction .	The reaction can be prolonged for up to 2hr.
	Insert fragment concentration is too low.	Increase the insert fragment concentration. (→See p4～6)
	Self-ligation of vector	Use dephosphorylated vector.
Excess number of colonies	Self-ligation of vector	Use dephosphorylated vector.
Freezing of the reagent	Storage conditions	In the case of -30℃ storage or long-term storage under -20℃, the reagent might freeze. No decrease in reaction efficiency is observed following 5 freeze-thaw cycles. .

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