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Product Information

QuickLink™ DNA Ligation Kit

Catalog Number **LIG2**
Storage Temperature –70 °C

TECHNICAL BULLETIN

Product Description

T4 DNA ligase is used for the joining of DNA molecules with compatible cohesive (sticky) termini, joining of blunt ended double stranded DNA molecules, and the repair of single stranded nicks in double stranded DNA.[1-3] The QuickLink™ DNA Ligation Kit has been optimized for utilization of T4 DNA ligase for rapid, efficient blunt and cohesive ligations performed at room temperature. The kit is used for the ligation of DNA fragments produced by restriction digestion, PCR, or other physical/enzymatic methods. It is designed for recircularization or cloning in plasmids and phage vectors, linker ligation, and for ligation product analysis by agarose gel electrophoresis.

Components

Sufficient reagents are provided for 50 ligation reactions.

T4 DNA Ligase 4 units/µl Catalog Number D2886	250 units
Ligation Buffer A (2×) Catalog Number L9537	500 µl
Ligation Buffer B (5×) Catalog Number L9662	100 µl

Reagents Required But Not Provided

- DNA to be ligated
- Water, Molecular Biology reagent grade, Catalog Number W4502

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

This kit is shipped in dry ice. Upon receipt, store kit at –70 °C. Thaw kit components before first use. After first usage, kit **must be stored at –20 °C** to avoid freeze-thaw cycles of the T4 DNA Ligase.

Procedure

The QuickLink DNA Ligation Kit has been optimized for maximum ligation and transformation yield. The DNA to be ligated should be highly purified, and dissolved in water or 10 mM Tris-HCl with 1 mM EDTA (TE buffer), pH 7.4–7.6. Ligation Buffer B is to be added **only** in cases in which the DNA is present in TAE. The TAE significantly decreases transformation efficiencies. Tris-borate-EDTA buffer (TBE) should not be used for DNA to be ligated.

Notes: While purifying the DNA, exposure of DNA to UV irradiation should be minimized in order to minimize the formation of pyrimidine dimers.

When performing a vector + insert ligation, it is recommended to dephosphorylate the vector DNA to minimize vector recircularization.

When the vector used is designed for blue-white selection (e.g., pBluescript®), it is recommended to coat the plates with Blue-White Select™ Screening Reagent (Catalog Number B3928) for the identification of white recombinant colonies/plaques harboring insert DNA.

T4 DNA Ligase can be inactivated by incubation at 70 °C for 10 minutes. For transformation assays, the T4 DNA ligase **should not** be inactivated.

If the total volume of DNA is greater than 10 µl, the volume of the ligation buffer and the ligase should be adjusted accordingly, and the incubation time should be extended to 20–30 minutes.

1. Thaw ligation buffer(s) (Catalog Number L9537 and/or L9662) on ice. Buffer solutions should be thoroughly mixed after thawing.

2. If the DNA to be ligated is in water, mix 10 µl of 2× Ligation Buffer A (Catalog Number L9537) and 10 µl of DNA for a total volume of 20 µl. If the DNA to be ligated is in Tris-acetate-EDTA (TAE) buffer, add 2 µl of 5× Ligation Buffer B (Catalog Number L9662) to 8 µl of DNA, then add 10 µl of 2× Ligation Buffer A for a total volume of 20 µl. Mix by finger tapping and then centrifuge tube briefly to bring down any droplets from the cap and sides of tube.

3. Add 1 µl of T4 DNA ligase (Catalog Number D2886) and mix thoroughly.

4. Incubate for 5 minutes at room temperature. Ligation can be extended up to 30 minutes.

5. Use immediately for transformation. Ligated DNA can be stored at –20 °C, but storage may reduce transformation efficiency.

Controls

Five control reactions are recommended, using the same amount of vector as in the ligation reaction for those controls with vector.

1. Vector only control **–** Perform transformation of the restricted vector (not ligated) in order to verify that all or almost all the vector is cut. The result of the transformation should be few or no colonies.

2. Insert only control **–** Perform transformation of the restricted insert in order to verify that the insert is not contaminated with vector DNA. The result of the transformation should be few or no colonies.

3. Vector dephosphorylation control **–** Perform ligation of the restricted vector after dephosphorylation and transformation of the ligation product. If the restricted vector is properly dephosphorylated, the result should be few or no colonies.

4. Competent cells controls:
   - Perform transformation of the competent cells with 5**–**10 ng of supercoiled plasmid, e.g., pUC19. This would give an estimation for the efficiency of the competent cells in use. The result should be at least 10^6 CFU/µg.
   - Perform transformation of competent cells with no vector. No colonies should appear, indicating that the selectable growth plate with the antibiotic is potent and that there is no contamination with other bacteria.

Recommended amounts and ratios of DNA to be ligated

Amounts – For plasmid ligation, the maximum amount of DNA to be ligated in 5 minutes should not exceed 200 ng. For phages, the amount of DNA to be ligated should be 500–1,000 ng.

Ratios – It is recommended that the ratio be optimized for each reaction. Note that higher ratios of insert DNA to vector DNA may result in multiple inserts.

- A molar ratio of insert DNA to vector of 3:1 is recommended. This ratio can vary from 2:1 to 5:1 for cohesive (sticky) end ligation and from 1:1 to 10:1 for blunt end ligation.
- For Bacteriophage lambda, a molar ratio of 1:1 for insert to phage arms is recommended. This ratio can vary from 0.125:1 to 4:1.
- A molar ratio of linker/adapter to vector in the range of 2:1 to 100:1 is recommended.

Cloning of PCR products

There are several methods for ligation of PCR products:

- Ligation of PCR product amplified by a polymerase leaving 3′ A-overhang, to a vector specified for it (AT-vector).
- Ligation of PCR product amplified with a polymerase leaving blunt ends, to a vector with blunt ends (not dephosphorylated).
- Ligation of phosphorylated PCR products to a dephosphorylated vector.
- Ligation of a restricted PCR product to a vector with compatible ends.
- It is possible to add the PCR product to the ligation mixture as is. In this case ligation can be performed for 5–30 minutes. Using purified PCR product will increase transformation yield.

Transformation

For transformation of competent cells produced by chemical treatment, a volume that does not exceed 1/10 the volume of competent cells is recommended. An excess of ligation reaction mixture may reduce the transformation efficiency.

Aliquots (1–2 µl) of the ligation reaction mixture may be used directly in electroporation. Otherwise, the sample should be desalted (ethanol precipitation is recommended) and then transformed.

References

1. Lehman, I.R., Science, **186**, 790 (1974).
2. Rossi, R., *et al*., Nucleic. Acids Res., **25**, 2106 (1997).
3. *Molecular Cloning, A Laboratory Manual*, 2nd ed., Sambrook, *et al.*, eds., Cold Spring Harbor Laboratory Press (Plainview, NY: 1989), pp. 1.53 – 1.59, 1.63-1.71, 2.94, 5.10-5.13, 5.61.
4. Hayashi, K. *et al*., Nucleic. Acids Res., **14**, 7617 (1986).

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Troubleshooting guide

Problem	Cause	Solution
Few or no colonies	Competent cells are not active.	Include competent cells control to confirm the efficiency of the cells (see first competent cell control).
	Growth medium or antibiotics are not suitable.	Use proper medium and antibiotics.
	Amount of DNA in the ligation reaction is not sufficient.	Check the DNA concentration. A sensitive method for measuring DNA concentration may be required, e.g., DNA Quantitation Kit, Catalog Number DNAQF.
	Ligation reaction did not work.	See problems and solutions for Poor ligation of PCR products.
Few or no **recombinant** colonies	Insert:vector ratio is not optimal.	Try to optimize insert:vector ratio starting with the suggested ratios.
	DNA ends are not compatible.	Make sure that the ends of the DNA are compatible. Repeat restriction and/or blunting (using fill-in and/or exonuclease). Check that the restriction enzymes used leave compatible ends.
	Restriction endonucleases are present, causing redigestion of the ligated products.	Purify the DNA. A phenol extraction may be performed, followed by an ethanol precipitation, and resuspension of the DNA in water or TE.
	Impurity due to purification method	Follow purification protocol accurately or consider another purification method. When performing phenol extraction, be sure no traces of phenol are present in the purified DNA preparation.
	The DNA has been purified by agarose gel electrophoresis.	Use high quality agarose, since some preparations may contain ligase inhibitors that are hard to remove.
Poor ligation of PCR products	Impure PCR product	Purify the PCR product.
	DNA ends are not compatible.	For PCR product harboring restriction endonuclease site, verify the number of bases 5′ to the restriction site is sufficient.
	Both vector and DNA fragment/oligonucleotide/ adapter/linker are not phosphorylated.	If the PCR product/oligonucleotide has not been ligated to a phosphorylated plasmid, phosphorylate the PCR product/oligonucleotide with T4 polynucleo-tide kinase and then perform a ligation reaction.
	Presence of an “extra” 3′ A on the PCR product from using *Taq* DNA polymerase.	Use an AT-vector or a vector that will accept the extra A.
	Absence of 3′ A on the PCR product from using a polymerase with 3′→5′ exonuclease activity.	Use a vector that does not require the “extra” 3′ A; do not use an AT-vector.