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ProductInformation

DNA LIGATION KIT

Product Code **LIG-1**

TECHNICAL BULLETIN

Product Description
One of the most important steps in the cloning process is the ligation of linear DNA into a cloning vector. This ability to join fragments of DNA through recombinant technology is essential for many basic experiments in biotechnology. Examples include protein expression, mutagenesis, gene analysis and structure-function relationships. DNA ligations are performed by incubating DNA fragments with appropriately linearized cloning vectors in the presence of buffer, ATP, and DNA ligase.

The DNA ligation kit contains the reagents necessary to increase the consistency of ligations. This kit contains T4 DNA ligase, the enzyme of choice for virtually all cloning purposes because of its ability to ligate both cohesive and blunt-ended strands of DNA. The kit also contains Polyethylene Glycol (PEG) 8000, which enhances blunt-ended ligations by macromolecular crowding. A ligation control DNA is included as a system check. The DNA Ligation Kit is application-tested and contains no detectable DNase activity.

Many parameters affect ligations such as the relative ratio of insert to vector, the quality and type of the DNA ends, the temperature of ligation and the concentration of DNA. Each of these factors need to be considered for a successful ligation.

Reagents Provided
Sufficient for 150 reactions

- 10X Ligation Buffer, Product No. D 2176 300 µl
  250 mM Tris-HCl, pH 7.8, 100 mM $MgCl_2$,
  10 mM dithiothreitol

- T4 DNA Ligase, Product No. D 2886 3 x 100 units
  1.0 U/:l in 50% glycerol containing
  10 mM Tris-HCl, pH 7.5, 50 mM KCl,
  1 mM dithiothreitol

- 10 mM ATP, Product No. A 3702 3 x 100 µl

- Control DNA, pBR322 DNA, HAE III Digest, 50 µl
  Product No. D 9430, 0.5 µg/µl in 10 mM Tris-HCl,
  pH 8.0, 1 mM EDTA

- 24% (w/v) PEG Solution, Product 1.5 ml
  No. P 2454

- Water, Molecular Biology Grade, 1.5 ml
  Product No. W 4502

Reagents Required But Not Provided
Vector DNA (0.033 µg/µl-1 :g/:l)
DNA fragment to be inserted (0.033 µg/µl-1 µg/µl)
Microcentrifuge tubes, 0.5 ml and 1.5 ml
A low temperature water bath may also be required

Abbreviations
ATP = Adenosine 5'-triphosphate
PEG = Polyethylene glycol 8000
DTT = Dithiothreitol
EDTA = Ethylenediaminetetraacetic acid

Precautions and Disclaimer
Sigma's DNA Ligation Kit is for laboratory use only. Not for drug, household, or other uses. Kit contains components that are hazardous. See the MSDS before using.

Storage
Store at –20 °C

Procedure
1. Remove kit reagents from freezer and place on ice bath to slowly thaw. Do not remove ligase from –20 °C until needed.

2. Determine the ratio of insert DNA to plasmid DNA. For most cloning applications, the molar ratio of insert to plasmid should be between 1 and 3. To achieve maximum ligation efficiency, it is recommended that a series of ligation reactions be set up, varying the ratio of insert DNA to plasmid DNA. (See Notes 1 and 7).

3. Prepare a 10 µl reaction mix by combining the following reagents:

0.02-1 µg	Vector DNA (See Notes 1, 6, and 7)
X µg	Insert DNA (See Notes 1 and 7)
1 µl	10X Ligation Buffer
1 µl	10 mM ATP Solution
X µl	Water to bring volume to 10 µl

Mix well and start reaction by adding

0.5-2 µl	T4 DNA Ligase (See Note 3)
10 µl	Total Volume

For the ligation of blunt-ended DNA, add 24% polyethylene glycol to a concentration of up to 15%, reduce ATP concentration to 0.5 mM, and add 10-fold excess or greater T4 ligase.[1,2] Addition of PEG also allows for more efficient ligation of lower concentrations of cohesive-ended DNA (See Note 4).

4. To assess the background due to incomplete restriction or dephosphorylation, include two negative control reactions, one without ligase added and one without insert added (See Note 6).

   To confirm performance of the reaction components, prepare a positive control ligation reaction to run concurrently with the experimental reaction. Substitute the vector DNA and insert DNA with 500 ng (1 µl) of Control DNA, and replace 5 µl of reaction (water) volume with 24% polyethylene glycol.

5. Mix thoroughly and incubate at 16 °C overnight (12 to 16 hours) (See Note 2).

6. When the incubation is completed, place on ice or store at –20 °C. If applicable, heat-inactivate at 65 °C for 10 minutes.

7. Evaluate only the positive control ligation reaction by agarose gel electrophoresis.

   Prepare a 1.0% (w/v) 1X TBE-buffered (Product No. T 6400) or 1X TAE-buffered (Product No. T 9650) agarose gel (Product No. A 9539) for electrophoresis.

   Add 2 µl of Gel Loading Solution (Product No. G 2526) to the positive control ligation reaction mix. Load entire volume onto the agarose gel. For comparison, load 0.1-0.2 µg of unligated Control DNA mixed with Gel Loading Solution.

   Run gel according to standard procedures and stain with ethidium bromide (Product No. E 1510). The ligation product will appear as a smear lying above the largest marker of the pBR322 Hae III Digest. Markers of the original digest may be minimally detectable.

Notes

1. The optimal insert to vector DNA ratio, is usually between 2:1 and 10:1. Higher concentrations of DNA reaction components will result in a higher rate of reaction. Ligation efficiency is also contingent on the integrity of the cohesive ends of the fragments being ligated.[1]

2. T4 DNA ligase is unstable at temperatures over 30 °C. Enzymatic activity diminishes with lower temperatures. An overnight incubation (10 to 16 hours) at 12-16 °C is effective for the broadest range of applications. Low temperatures favor the annealing of cohesive ends. However, room temperature is recommended for blunt-ended ligations. Typically, the lower incubation temperatures are used in conjunction with longer incubation times.[1]

3. The final concentration of glycerol (a component of the ligase storage buffer) must not exceed 5% in the final reaction mixture.[2]

4. The addition of PEG to the reaction mixture facilitates the ligation of blunt-ended fragments. Also, it allows lower concentrations of cohesive-ended DNA to be ligated. Under these conditions, ligation of cohesive-ended fragments can be stimulated 10- to 100-fold by addition of PEG to a final concentration of 15% in the reaction mixture. The concentration of PEG in a reaction mixture should not exceed 15% because at higher concentrations, tandem concatemers may became a major reaction product. Also, it has been observed that the ligation of lambda DNA in the presence of 12% PEG can result in large concatemers that may precipitate from the reaction.[1,2]

5. T4 DNA Ligase is not inhibited by the presence of tRNA, but is strongly inhibited by NaCl at concentrations of 150 mM or greater.

6. When a vector is digested with a single restriction endonuclease, it can potentially self-ligate. Removal of the 5'-phosphates from the digested vector, using alkaline phosphatase (Product No. P 4978) will prevent self-ligation. Dephosphorylated vector will ligate only to a DNA insert possessing 5'-phosphate moieties, forming two phosphodiester bonds. The two remaining phosphodiester bonds are repaired following transformation of the vector:insert into a competent host.

7. Use the formula below to calculate the concentration of ends (in picomoles) to be ligated per µg of DNA fragments:

$$\frac{picomoles\ of\ ends}{mg\ DNA} = \frac{10^{-6}\ g\ DNA}{(\#\ base\ pairs)(660\ g/mole\ base\ pair)}\ x\ 10^{12}\ picomoles/mole\ x\ 2\ ends\ per\ DNA\ fragment$$

References

1. Ausubel, F. M., *et al*., Current Protocols in Molecular Biology, John Wiley and Sons, Inc., Volume 1, 3.14.1-3.16.11 (1994)
2. Sambrook, J., *et al*., Molecular Cloning: A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 1.63-1.71 (1989)