DNA Ligation Kit

Instruction Manual

Catalog #203003

Revision C0

For Research Use Only. Not for use in diagnostic procedures.

203003-12

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DNA Ligation Kit

CONTENTS

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MATERIALS PROVIDED

Note *Aliquot the 10 mM rATP into smaller volumes after initial thawing to avoid multiple freeze–thaw cycles.*

Materials provided	Quantity	Storage temperature
10 mM rATP (pH 7.5) in sterile water	4 × 250 µl (1 ml total volume)	–20°C [a]
cI857 wild-type lambda control DNA, *Hin*d III digested	10 µg	–20°C
pUC18 plasmid control DNA, *Bam*H I digested	10 µg	–20°C
T4 DNA ligase (4 U/µl)	300 U	–20°C
10× ligase buffer[b]	1 ml	–20°C

[a] For long-term storage, store at –80°C.

[b] See *Preparation of Media and Reagents*.

STORAGE CONDITIONS

10 mM rATP (pH 7.5): –20°C (–80°C for long-term storage)

***c*I857 Wild-Type Lambda Control DNA**: –20°C

pUC18 Plasmid Control DNA: –20°C

T4 DNA Ligase: –20°C

10× Ligase Buffer: –20°C

INTRODUCTION

The DNA Ligation Kit contains the reagents necessary to perform both sticky and blunt-end ligations. These reagents are guaranteed to be DNase free while providing optimal ligation efficiency. The kit contains reagents for 150 ligations and is optimized for use with Agilent's lambda vector arms and plasmid vectors.

Revision C0

PREPROTOCOL CONSIDERATIONS

- When setting up ligations, **gently** mix the ligation reaction with the pipet tip after adding the ligase.
- T4 DNA ligase is not inhibited by transfer RNA (tRNA), but is strongly inhibited by NaCl concentrations >150 mM. To remove NaCl, wash DNA pellets with 80% (v/v) alcohol prior to resuspension.
- The efficiency of any ligation reaction depends on the integrity of the cohesive ends being ligated and on the quality of the vector DNA.
- Plasmid blunt-end ligations may be enhanced by reducing the final rATP concentration by half and lengthening the ligation time.
- When performing any ligation experiment, it is important to include a control ligation of the prepared vector alone. This will provide a good evaluation of the background associated with the vector in the experiment.

  The following formula allows easy calculation of picomoles per end:

$$\frac{2 \times 10^6}{660 \times \text{number of base pairs}} = \text{picomole ends per microgram of DNA}$$

PROTOCOL

Ligation of Insert DNA into Lambda Vector Arms

Control Ligation

To verify the activity of the components, perform the following control ligation using 1 µl of the *c*I857 wild-type lambda control DNA.

1. Add the following components to a microcentrifuge tube:

   1 µg (1–2 µl) of *c*I857 wild-type lambda control DNA
   0.5 µl of 10× ligase buffer
   0.5 µl of 10 mM rATP (pH 7.5)
   X µl of sterile water for a final volume of 4.5 µl

   Then add

   0.5 µl of T4 DNA ligase (4 U/µl)

   Note *Keep the final glycerol content of the T4 DNA ligase below 5%.*

2. Incubate the control ligation reaction at 12°C for 4–6 hours or at 4°C overnight.

3. Prepare a 0.6% (w/v) agarose gel and load 1 µl of unligated *c*I857 wild-type lambda control DNA and 5 µl of the control ligation reaction. After running the gel, compare the unligated lanes with the ligated lanes to verify evidence of activity.

Sample Ligation

1. Add the following components to a microcentrifuge tube:

   1 µg of prepared lambda vector arms
   X µg of insert DNA[‖]
   0.5 µl of 10× ligase buffer
   0.5 µl of 10 mM rATP (pH 7.5)
   X µl of sterile water for a final volume of 4.5 µl

   Then add

   0.5 µl of T4 DNA ligase (4 U/µl)

   Note *Keep the final glycerol content of the T4 DNA ligase below 5%.*

2. Incubate the sample ligation reaction at 12°C for 4–6 hours or at 4°C overnight.

[‖] When ligating into lambda vector arms, we recommend using an equimolar insert-to-vector ratio in order to prevent multiple inserts.

Ligation of Insert DNA into Plasmid Vectors

Control Ligation

To verify the activity of the components, perform the following control ligation using 1 µl of pUC18 plasmid control DNA.

1. Add the following components to a microcentrifuge tube:

   1 µg (1–2 µl) of pUC18 plasmid control DNA
   1 µl of 10× ligase buffer
   0.5 µl of 10 mM rATP (pH 7.5)
   X µl of sterile water for a final volume of 9.5 µl

   Then add

   0.5 µl of T4 DNA ligase (4 U/µl)

   Note *Keep the final glycerol content of the T4 DNA ligase below 5%.*

2. Incubate the control ligation reaction at 12°C for 4–6 hours or at 4°C overnight.

3. Prepare a 0.6% (w/v) agarose gel and load 1 µl of unligated pUC18 plasmid control DNA and 10 µl of the control ligation reaction. After running the gel, compare the unligated lanes with the ligated lanes to verify evidence of activity.

Sample Ligation

1. Add the following components to a microcentrifuge tube:

   X µg of vector DNA
   X µg of insert DNA[||]
   1 µl of 10× ligase buffer
   1 µl of 10 mM rATP (pH 7.5)
   0.5 µl of T4 DNA ligase (4 U/µl)
   X µl of sterile water for a final volume of 10 µl

   Note *Keep the final glycerol content of the T4 DNA ligase below 5%.*

2. Incubate the sample ligation reaction at 12°C for 4–6 hours or at 4°C overnight.

[||] Each vector–insert combination requires unique ligation conditions. To obtain optimum results, we recommend testing various vector-to-insert ratios to ensure the highest ligation efficiency (e.g., insert-to-vector ratios from 2:1 to 10:1).

PREPARATION OF MEDIA AND REAGENTS

10× Ligase Buffer

500 mM Tris-HCl (pH 7.5)
70 mM $MgCl_2$
10 mM dithiothreitol (DTT)

Note *rATP is added separately in the ligation reaction*

MSDS INFORMATION

Material Safety Data Sheets (MSDSs) are provided online at *http://www.genomics.agilent.com*. MSDS documents are not included with product shipments.