For life science research only.
Not for use in diagnostic procedures.

Rapid DNA Ligation Kit

Version: 12
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Standard assay: 50 ng linearized and dephosphorylated vector DNA and 150 ng insert DNA.

Cat. No. 11 635 379 001 1 kit
40 reactions

Store the kit at −15 to −25°C.

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1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	Rapid DNA Ligation Kit, T4 DNA Ligation Buffer, 2x conc.	For ligation reaction.	1 vial, 0.5 ml
2	Rapid DNA Ligation Kit, DNA Dilution Buffer, 5x conc.	To prepare DNA for ligation.	1 vial, 0.5 ml
3	Rapid DNA Ligation Kit, T4 DNA Ligase	5 U/µl	1 vial, 40 µl

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the kit is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	T4 DNA Ligation Buffer, 2x conc.	Store at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***
2	DNA Dilution Buffer, 5x conc.	
3	T4 DNA Ligase	

1.3. Additional Equipment and Reagent required

- Competent Cells

For Electroporation

- Electroporation unit, such as a BioRad pulsar unit
- High Pure PCR Product Purification Kit*
- Electrocompetent cells

1.4. Application

The Rapid DNA Ligation Kit can rapidly ligate DNA with either blunt or sticky ends. Depending on the DNA concentration in the reaction, the ligation products will be either circular (if the DNA concentration is low) or concatemeric (if the DNA concentration is high).

The kit can be used for:
- Cloning fragments into either plasmid or phage vectors
- Linker ligation
- Recircularization of linear DNA

⚠ ***Electroporation can be performed after ligation in combination with the High Pure PCR Product Purification Kit.***

After the DNA is ligated, it may be purified with the High Pure PCR Product Purification Kit. Purified, ligated DNA may be introduced into cells by electroporation.

The kit contains all reagents necessary for ligation. No additional reagents or additives are required.

1.5. Preparation Time

Assay Time

Five minutes for sticky-end or blunt-end ligations at +15 to +25°C.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Dephosphorylated DNA with either blunt or sticky ends.

General Considerations

Standard DNA Ligation Reaction

For optimal results, follow these guidelines.

Step	Recommendation
Purification	Prior to ligation, purify the DNA with either the High Pure PCR Product Purification Kit or phenol extraction and ethanol precipitation.
Dephosphorylation	For insertion of DNA into plasmid vectors, dephosphorylate the vector DNA with Alkaline Phosphatase* (unless it will be recircularized).
Reaction Volume	▪ To prepare the DNA for ligation, dissolve the DNA in 1x concentrated DNA Dilution Buffer (prepared from Vial 2) to make a total volume of 10 µl. ▪ If the total volume of DNA solution in the 1x DNA Dilution Buffer is greater than 10 µl, increase the volume of all other reagents in the reaction accordingly and incubate the ligation reaction for 30 minutes instead of 5 minutes.
Molar Ratio	▪ The molar ratio of vector DNA to insert DNA in the standard ligation reaction should be 1:3, for example, 50 ng linearized, dephosphorylated plasmid vector DNA plus 150 ng insert DNA (if the vector and insert DNA are approximately the same length). ▪ Alternatively, if the vector DNA and insert DNA are not similar in length, use a 1:1 or 1:2 molar ratio of vector to insert. ▪ A molar ratio of 1:5 can be used for sticky-end ligations. ⚠ ***However, if a 1:5 ratio (vector:insert) is used for blunt-end ligations, the resulting product will generate fewer transformed colonies.***
Transformation	To avoid inhibiting the transformation reaction with surplus DNA, use no more than 1/10 of the ligation reaction mixture in the transformation.
Maximum Amount of DNA	The maximum amount of DNA to be ligated in 5 minutes should not exceed 200 ng.
T4 DNA Ligase Inactivation	T4 DNA Ligase can be completely inactivated by a 10 minute incubation at +65°C. Inactivation is necessary only if the ligation reaction mixture is to be used in experiments other than transformation assays. ⚠ ***Heat inactivation of the ligation reaction mixture before transformation causes the number of transformed colonies to decrease drastically (> a factor of 20).***

Ligation Reaction for Insertion into Phage Vectors (Including Linker Ligation)

For optimal results, follow these guidelines.

Step	Recommendation
Purification	Prior to ligation, purify the DNA with either the High Pure PCR Product Purification Kit or by phenol extraction and ethanol precipitation.
Dephosphorylation	For insertion of DNA into phage vectors, dephosphorylate the arms of the vector DNA with Alkaline Phosphatase*.
Reaction Volume	If the total volume of DNA solution in the 1x DNA Dilution Buffer is greater than 10 µl, increase the volume of all other reagents in the reaction accordingly and incubate the ligation reaction for 30 minutes instead of 5 minutes.
Ratio of Vector Arms to DNA	The ratio of vector arms to insert DNA should be approximately 8:1, for example, 1,000 ng DNA, lambda gt 11, *Eco R*I arms (dephosphorylated), plus 120 ng insert DNA in a total reaction volume of 10 µl.
T4 DNA Ligase Inactivation	T4 DNA Ligase can be completely inactivated by a 10 minute incubation at +65°C. Inactivation is necessary only if the ligation reaction mixture is to be used in experiments other than packaging assays. ⚠ ***Heat inactivation of the ligation reaction mixture before packaging drastically decreases the number of plaques formed.***

Working Solution

Preparation of Rapid DNA Ligation Kit Working Solution

To prepare the 1x concentrated DNA Dilution Buffer, thoroughly mix the DNA Dilution Buffer, 5x conc. (Vial 2); then dilute it fivefold with autoclaved double-distilled water.

2.2. Protocols

Standard DNA Ligation Reaction

For standard ligation reactions, follow the protocol below.

1. Dissolve vector DNA and insert DNA into thoroughly mixed and diluted 1x concentrated DNA Dilution Buffer (see ***Working Solution***) to a final volume of 10 µl in a autoclaved reaction vial.

2. Thoroughly mix T4 DNA Ligation Buffer (Vial 1).
   ⚠ ***Always mix the contents of Vial 1 immediately before using.***

   – Add 10 µl T4 DNA Ligation Buffer (Vial 1) to the reaction vial and mix thoroughly.

3. Add 1 µl T4 DNA Ligase (Vial 3) and mix thoroughly.

4. Incubate for 5 minutes at +15 to +25°C.

5. The ligation reaction mixture can be used directly for the transformation of competent cells, or can be stored without heat inactivation at −15 to −25°C.
   ⚠ ***Heat inactivation of the T4 DNA Ligase drastically decreases the transformation efficiency.***
   ⚠ ***Do not use more than 1/10 of the volume of the ligation reaction mixture for the transformation assay.***

6. The ligated DNA can be analyzed by agarose gel electrophoresis.

Electroporation of *E. coli* Cells after Recircularization

Preparation of Working Solutions from the High Pure PCR Product Purification Kit

The Wash Buffer (Vial 2) must contain ethanol to be effective. Make sure you have added the appropriate amount of ethanol p.a. to the Wash Buffer prior to use. Add either 40 ml ethanol to the buffer in the smaller (50 purification) kit or 200 ml ethanol to the buffer in the larger (250 purification) kit.

⚠ ***The Binding Buffer (Vial 1) contains guanidine-HCl which is an irritant. Wear gloves and follow standard safety precautions when handling the Binding Buffer.***

Preliminary DNA Purification

Before using the ligated DNA for electroporation, purify the DNA according to the following procedure.

⚠ ***The use of the High Pure PCR Product Purification Kit adds only 10 to 15 minutes to the overall time required to prepare electrocompetent reactions.***

1. Add 100 µl Binding Buffer (Vial 1) to the 20 µl ligation reaction mixture.

2. Pipette the sample into the upper reservoir of a combined Filter Tube-Collection Tube assembly.

3. Centrifuge 1 minute at maximum speed in a standard tabletop centrifuge.

4. Discard the flow through.
   – Reinsert the filter tube into the collection tube.

5. Add 500 µl Wash Buffer (Vial 2) to the upper reservoir.
   – Centrifuge 1 minute at maximum speed.
   ⚠ ***Make sure that the filter tube does not touch the surface of the Wash Buffer flow through.***

6. Discard the flow through.
   – Reinsert the filter tube into the collection tube.
   – Add 200 µl Wash Buffer (Vial 2) to the upper reservoir.
   – Centrifuge 1 minute at maximum speed (13,000 rpm).

7. Discard the flow through.
   – Reinsert the filter tube into the collection tube.
   – Centrifuge 1 minute at maximum speed to remove residual Wash Buffer.

8. Discard the collection tube.
   – Insert the filter tube into a clean 1.5 ml reaction tube.

9. Add 100 µl autoclaved double-distilled water (pH approx. 7.4) to the upper reservoir.
   – Centrifuge 1 minute at maximum speed.

10. Save the flow through as it contains the ligation product. The final volume recovered is approximately 100 µl.
    – Use 10 µl of the flow through (containing one-tenth of the ligation product) in the electroporation procedure.

Electroporation

1. Electroporate 1/10 volume of the ligation mixture into electrocompetent cells using the BioRad pulsar unit and 0.2 cm cuvettes.
   – Use the following power settings during the procedure: 2.5 kV, 25 MF, 200 ohm.
   – The specific electroporation settings must be determined for each strain.

2. Plate out 1/20 volume of the electroporated cells.
   Colonies on each plate represent the yield from 0.5 ng DNA.

Ligation Reaction for Insertion into Phage Vectors (Including Linker Ligation)

Use the following modified ligation protocol to insert DNA into phage vectors.
If the ligation requires an adaptor, add it in Step 1.

1. In a autoclaved reaction vial, dissolve a mixture of DNA [vector arms + insert + adaptor (if needed)] in enough 1x concentrated DNA Dilution Buffer to make a final volume of 10 µl.

2. Add 10 µl T4 DNA Ligation Buffer, 2x conc. (Vial 1) and mix thoroughly.
   Always mix the contents of Vial 1 immediately before use.

3. Add 1 µl T4 DNA Ligase (Vial 3) and mix thoroughly.

4. Incubate for 5 minutes at +15 to +25°C.

5. For each packaging reaction, use 4 µl of the ligation reaction mixture. The ligation reaction mixture can be stored without heat inactivation at −15 to −25°C.
   Heat inactivation of the T4 DNA Ligase drastically decreases the packaging efficiency.

3. Results

In a typical experiment, a ligation reaction involving 1 µg of vector DNA will produce the following results:

Recircularization	**pUC19/*Sma* I**	**pUC19/*Hind* III**
Yield of transformed colonies	>1 × 10^6	>1 × 10^6
Insertion of Insert Into Plasmid Vectors	**pUC19/*Sma* I + 2,100 bp Insert**	**pUC19/*Hind* III + 2,300 bp Insert**
Yield of white colonies after transformation into competent *E. coli* JM83 cells	>1 × 10^5	>6 × 10^5
Insertion of DNA Into Phage Vectors	**Lambda gt 11 Arms + Insert/*Eco* RI**	
Yield of white plaques	>1 × 10^7	

Result Analysis

To analyze the products from the DNA ligation reaction by agarose gel electrophoresis, add 1/5 volume of gel loading buffer (1% SDS (w/v), 50 mM EDTA, 0.02% bromphenol blue (w/v), 50% glycerol (v/v), pH 7.5.) to an aliquot of the ligation product (½ of the plasmid ligation product or ¼ of the phage ligation product). For example, add 2 µl gel loading buffer to 10 µl plasmid ligation product.

⚠ ***If you want to see ligated, circular DNA on an agarose gel, you must start with enough DNA in the original ligation reaction. However, the concentration of the DNA in the ligation reaction should never be more than 10 ng DNA per µl of reaction mixture (200 ng/20 µl standard reaction). If you need to ligate more than 200 ng DNA, increase the ligation reaction volume (and ligation time).***

Recircularization

Fifty nanogram pUC19 DNA, digested with either *Sma I* or *Hind* III, was then religated according to the standard protocol and transformed into competent *E. coli* JM83 cells. The yield of transformed colonies per mg of DNA is shown:

pUC19 DNA Undigested	**pUC19/*Sma* I Digest**	**pUC19/*Hind* III Digest**
2 × 10^7	7 × 10^6	8 × 10^6

Cloning of an Insert Into Plasmid Vectors

Fifty nanogram pUC19 DNA, digested with either *Sma* I or *Hind* III, then dephosphorylated with Alkaline Phosphatase*, was ligated with 150 ng either *Sma* I or *Hind* III DNA fragments. The yield of white colonies per mg of vector DNA after transformation into competent *E. coli* JM83 cells is shown:

pUC19 DNA Undigested	**pUC19/*Sma* I Digest + 2,100 bp Insert DNA**	**pUC19/*Hind* III Digest + 2,300 bp Insert DNA**
2 × 10^7	6 × 10^5	1.3 × 10^6

Cloning of an Insert Into Phage Vectors

One microgram of DNA, lambda gt 11, *Eco*R I arms (dephosphorylated), was ligated to 120 ng pUC19 DNA, which was linearized with either *Sma* I or *Eco*R I. For ligation of blunt ends, 20 ng of an adaptor was added. After the ligated products were packaged into phages, the phages were used to infect *E. coli* Y1090 cells. The cells were then plated onto agar.
The yield of white plaques is shown:

Lambda gt 11 Vector Control	**Lambda gt 11 Arms + Insert/*Sma* I + Adaptor**	**Lambda gt 11 Arms + Insert/*Eco*R I**
1.6×10^9	4.9×10^4	1.2×10^8

4. Troubleshooting

Observation	Recommendation	
If a cloning/transformation experiment does not turn out as expected, use the controls to identify the problem.	**Controls**	**Result Shows**
	Transformation of uncut vector DNA (*e.g.*, 50 picograms).	Transformation efficiency of cells per microgram of DNA.
	Transformation of linearized unligated vector DNA.	Completeness of restriction enzyme cleavage.
	Transformation of religated dephosphorylated vector.	Efficiency of dephosphorylation.
	Transformation of linearized and recircularized vector.	Efficiency of ligation.
	Transformation without DNA (*e.g.,* with DNA buffer alone).	Control for competent cells, that is, no growth indicates that the cells are not contaminated or already contain a plasmid. Growth on a selective medium indicates that the cells are contaminated and already contain a plasmid.

5. Additional Information on this Product

5.1. Quality Control

Ligation and Recutting

1 µg pUC19 DNA, digested with *Hind* III, is dephosphorylated with Alkaline Phosphatase, then ligated with 1 µg of DNA Molecular Weight Marker II, according to the standard protocol. After ethanol precipitation, the ligation products are redigested with *Hind* III. When the digestion products were analyzed on an agarose gel, only the original digestion pattern was seen.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Layout changes.
Editorial changes.

6.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Alkaline Phosphatase	1,000 U, 1 U/µl	10 713 023 001
	1,000 U, 20 U/µl	11 097 075 001
High Pure PCR Product Purification Kit	1 kit, up to 50 purifications	11 732 668 001
	1 kit, up to 250 purifications	11 732 676 001

6.4. Trademarks

All product names and trademarks are the property of their respective owners.

6.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

6.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

6.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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