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Rapid DNA Ligation Kit

Cat. No. 11 635 379 001

Kit for 40 ligation reactions

Version 11
Content version: May 2019
Store at −15 to −25°C

1. What this Product Does

Number of Tests

40 ligation reactions (standard assay: 50 ng linearized and dephosphorylated vector DNA and 150 ng insert DNA).

Kit Contents

Vial	Label	Contents
1	T4 DNA Ligation Buffer, 2x conc.	0.5 ml
2	DNA Dilution Buffer, 5x conc.	0.5 ml
3	T4 DNA Ligase	• 40 µl • [5 U/µl]

Storage and Stability

The unopened kit is stable at –15 to -25°C until the expiration date printed on the label.

⚠ Avoid repeated freezing and thawing.

Additional Equipment /Reagents Required

- Competent Cells

For Electroporation:

- Electroporation unit, *e.g.* BioRad pulsar unit
- High Pure PCR Product Purification Kit*
- Electrocompetent cells

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).

For example, 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 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.

Assay time

5 min ligation (for sticky-end or blunt-end ligation at +15 to +25°C)

2. How to Use This Product

2.1 Standard Ligation Reaction for DNA

Before You Begin

For optimal results, follow these guidelines:

Step	Recommendation
Purification	Before it is ligated, the DNA should be purified, either with the High Pure PCR Product Purification Kit* or by phenol extraction and ethanol precipitation.
Dephosphorylation	For insertion of DNA into plasmid vectors the vector DNA should be dephosphorylated with Alkaline Phosphatase* (unless it is to be recircularized).
Reaction volume	• To prepare the DNA for ligation, dissolve it in 1x concentrated DNA Dilution Buffer (prepared from kit vial 2) to make a total volume of 10 µl. • If the total volume of DNA solution in 1x DNA Dilution Buffer is greater than 10 µl, then increase the volume of all other reagents in the reaction accordingly and incubate the ligation reaction for 30 min (instead of 5 min).
Molar ratio	• The molar ratio of vector DNA to insert DNA the standard ligation reaction should be 1:3, e.g. 50 ng linearized, dephosphorylated plasmid vector DNA plus 150 ng insert DNA (if the vector and insert DNA are approx. the same length). • Alternatively, if the vector DNA and insert DNA are not similar in length, you may 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 ligation, 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 min should not exceed 200 ng.
T4 DNA Ligase inactivation	T4 DNA ligase* can be completely inactivated by a 10 min 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 (> factor of 20).

Sample material

Dephosphorylated DNA with either blunt or sticky ends.

** available from Roche Diagnostics*

Preparation of Kit Working Solutions

Prepare 1x conc. DNA Dilution Buffer by mixing 5x conc. DNA Dilution Buffer (vial 2) thoroughly, then diluting it fivefold with double dist. water.

Procedure

For standard ligation reactions, follow the procedure below.

❶	Dissolve vector DNA and insert DNA in thoroughly mixed and diluted **1 x conc. DNA Dilution Buffer** to a final volume of 10 µl in a sterile reaction vial.
❷	• Mix thoroughly **T4 DNA Ligation Buffer** (vial 1). ⚠ You must always mix the contents of vial 1 immediately before using it. • Add 10 µl **T4 DNA Ligation Buffer** (vial 1) to the reaction vial. • Mix thoroughly.
❸	• Add 1 µl **T4 DNA Ligase** (vial 3). • Mix thoroughly.
❹	Incubate for 5 min at +15 to +25°C.
❺	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.
❻	The ligated DNA can be analyzed by agarose gel electrophoresis.

2.2 Electroporation of *E.coli* cells after recircularization

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

The Wash Buffer (blue cap) must contain ethanol to be effective. Make sure you have added the appropriate amount of ethanol p.a. to the Wash Buffer before you use it. You 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.

Caution

The Binding Buffer (green cap) contains guanidine-HCl, which is an irritant. Wear gloves and follow usual safety precautions when handling.

Procedures

Preliminary purification

Before using the ligated DNA for electroporation, you should use the procedure below to purify it.

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

❶	Add 100 µl **Binding Buffer** to the 20 µl ligation reaction mixture.
❷	Pipet the sample into the upper reservoir of a combined Filter Tube-Collection Tube assembly.
❸	Centrifuge 1 min at max. speed in a standard table top centrifuge.
❹	• Discard the flowthrough. • Reinsert the filter tube in the collection tube.
❺	• Add 500 µl **Wash Buffer** (blue cap) to the upper reservoir. • Centrifuge 1 min at max. speed. ⚠ Make sure that the filter tube does not touch the surface of the Wash Buffer flowthrough.
❻	• Discard the flowthrough. • Reinsert the filter tube in the collection tube. • Add 200 µl **Wash Buffer** (blue cap) to the upper reservoir. • Centrifuge 1 min at max. speed (13 000 rpm).
❼	• Discard the flowthrough. • Reinsert the filter tube in the collection tube. • Centrifuge 1 min at max. speed to remove residual Wash Buffer.
❽	• Discard the collection tube. • Insert the filter tube in a clean 1.5 ml reaction tube.
❾	• Add 100 µl **sterile double dist. water** (pH approx. 7.4) to the upper reservoir. • Centrifuge 1 min at max. speed
❿	• Save the flowthrough; it contains the ligation product. The final volume recovered is about 100 µl. • Use 10 µl of the flowthrough (containing one-tenth of the ligation product) in the electroporation procedure below.

Electroporation

- 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.)

- Plate out 1/20 volume of the electroporated cells.

Colonies on each plate represent the yield from 0.5 ng DNA.

2.3 Ligation Reaction for Insertion into Phage Vectors (including Linker Ligation)

Before You Begin

For optimal results, follow these guidelines:

Step	Recommendation
Purification	Before it is ligated, the DNA should be purified, either with the High Pure PCR Product Purification Kit* or by phenol extraction and ethanol precipitation.
Dephosphorylation	For insertion of DNA in phage vectors, the arms of the vector DNA should be dephosphorylated with alkaline phosphatase.
Reaction volume	If the total volume of DNA solution in 1× DNA Dilution Buffer is greater than 10 µl, then increase the volume of all other reagents in the reaction accordingly and incubate the ligation reaction for 30 min (instead of 5 min).
Ratio of vector arms to DNA	The ratio of vector arms to insert DNA should be approx. 8:1 (*e.g.* 1000 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 min 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.

Procedure

Use the modified ligation procedure below to insert DNA into phage vectors.

⚠ If the ligation requires an adaptor, add it in step 1 below.

❶	In a sterile 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.
❷	• Add 10 µl **T4 DNA Ligation Buffer** (2 × conc.) (vial 1). ⚠ You must always mix the contents of vial 1 immediately before using it. • Mix thoroughly.

❸	• Add 1 µl **T4 DNA Ligase** (vial 3). • Mix thoroughly.
❹	Incubate for 5 min at +15 to +25°C.
❺	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. Typical 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	> 1x10^6	> 1x10^6

Insertion of an insert in plasmid vectors	pUC19/*Sma* I + 2100 bp insert	pUC19/*Hind* III + 2300 bp insert
Yield of white colonies after transformation into competent E. coli JM83 cells	> 1x10^5	> 6x10^5

Insertion of DNA into phage vectors	lambda gt 11 arms + insert/*Eco* RI
Yield of white plaques	>1x10^7

Analysis of results

To analyze the products from the DNA ligation reaction by agarose gel electrophoresis, add 1/5 volume of gel loading buffer (below) to an aliquot of the ligation product (1/2 of the plasmid ligation product or 1/4 of the phage ligation product), e.g. add 2 µl gel loading buffer to 10 µl plasmid ligation product.

Gel loading buffer:

1% SDS (w/v), 50 mM EDTA, 0.02% bromophenol blue (w/v), 50% glycerol (v/v), pH 7.5.

⚠ 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

50 ng pUC19 DNA, digested with either *Sma I* or *Hind* III, was then religated according to the standard protocol and transformed into competent [Hanahan method (3)] *E. coli* JM83 cells.

Yield of transformed colonies per mg of DNA:

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

50 ng 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.

Yield of white colonies per mg of vector DNA after transformation into competent *E. coli* JM83 cells:

pUC19 DNA undigested	pUC19/*Sma* I digest + 2100 bp insert DNA	pUC19/*Hind* III digest + 2300 bp insert DNA
2 x10^7	6 x10^5	1.3 x10^6

Cloning of an insert into phage-vectors

1 µg of DNA, lambda gt 11, *Eco*R I arms (dephosphorylated), was ligated to 120 ng pUC19 DNA, which had been linearized with either *Sma* I or *Eco*R I. (For ligation of blunt ends, 20 ng of an adaptor were 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.

Yield of white plaques:

lambda gt 11 vector control	lambda gt 11 arms + insert/*Sma* I + adaptor	lambda gt 11 arms + insert/*Eco*R I
1.6 x10^9	4.9 x10^4	1.2 x10^8

4. Troubleshooting

If a cloning/transformation experiment does not turn out as expected use the following controls to determine what went wrong:

Control	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, i.e. 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 References

1 Hayashi, K. et al. (1986) *Nucleic Acids Res.* **14**, 7617–7631.

2 Sambrook, J..,Fritsch, E. F. & Maniatis, T (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, (NY): 1989, 2nd ed., Vol 1,2 and 3,p 561–563.

3 Hanahan, D. (1983) *J. Mol. Biol.* **166**, 557–580.

4 Easy Dephosphorylation and Rapid DNA Ligation (2003) *Biochemica* **4**, 23.

5 Chen, X.-S. & Funk, C.D. (2001) The N-terminal "-Barrel" Domain of 5-Lipoxygenase Is Essential for Nuclear Membrane Translocation. *J. Biol. Chem.* **276,** 811-818.

6 Escher, P. et al (2001) Rat PPARs: Quantitative Analysis in Adult Rat Tissues and Regulation in Fasting and Refeeding. *Endocrinology* **142,** 4195-4202.

7 Someya, A. et al (2001) ARF-GEP100, a guanine nucleotide-exchange protein for ADP-ribosylation factor, *Proc Natl Acad Sci USA.* **27,** 98(5): 2413–2418.

Please refer to our website for the following information:

8 Lab FAQS

9 Restriction Enzymes Frequently Asked Questions and Ordering Guide

10 Molecular Weight Markers for Nucleic Acids

11 PCR Product Selection Guide

5.2 Quality Control

Ligation and Recutting

1 µg pUC19 DNA, digested with *Hind* III, was 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 were 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

Text Conventions
To make information consistent and memorable, the following text conventions are used in this document:

Text Convention	Usage
Numbered instructions labeled ❶, ❷ etc.	Steps in a procedure that must be performed in the order listed.
Asterisk *	Denotes a product available from Roche Diagnostics.

Symbols
In this document, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
	Important Note: Information critical to the success of the procedure or use of the product.

6.2 Ordering Information

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Product	Pack Size	Cat. No.
Blue / White Selection		
X-gal (5-Bromo-4 chloro-3-indolyl-β-D-galactopyranoside)	100 mg 250 mg 1 g 2.5 g	11 680 293 001 10 651 745 001 10 745 740 001 10 703 729 001
Isopropyl-β-D-thiogalactoside (IPTG)	1 g 5 g	10 724 815 001 11 411 446 001
Ampicillin	5 g 50 g	10 835 242 001 10 835 269 001
Dephosphorylation		
Alkaline Phosphatase, Shrimp	1000 U	11 758 250 001
Alkaline Phosphatase, molecular biology quality	1000 U (1 U/µl) 1000 U (20 U/µl)	10 713 023 001 11 097 075 001
Gel Electrophoresis		
Agarose MP	100 g 500 g	11 388 983 001 11 388 991 001
Restriction Enzymes	*Please refer to website or catalogue*	
Water, PCR Grade, specially purified, double-distilled, deionized and autoclaved	100 ml (4 vials of 25 ml) 25 ml (25 vials of 1 ml) 25 ml (1 vial of 25 ml)	03 315 843 001 03 315 932 001 03 315 959 001
Ligation		
T4 DNA Ligase	100 U [1 U/µl] 500 U [1 U/µl] 500 U [≥5 U/µl]	10 481 220 001 10 716 359 001 10 799 009 001
Nucleic Acid Purification		
Agarose Gel DNA Extraction Kit	1 kit (max. 100 reactions)	11 696 505 001
High Pure PCR Product Purification Kit	50 purifications 250 purifications	11 732 668 001 11 732 676 001
High Pure Plasmid Isolation Kit	50 purifications 250 purifications	11 754 777 001 11 732 676 001

Changes to previous version
Editorial changes.

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