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

Rapid DNA Dephos & Ligation Kit

Version: 05
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Kit for fast and efficient dephosphorylation and ligation of sticky- or blunt-end DNA fragments.

Cat. No. 04 898 117 001	1 kit 40 reactions
Cat. No. 04 898 125 001	1 kit 160 reactions

Store the kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Catalog number	Content
1	Rapid DNA Dephos & Ligation Kit, rAPid Alkaline Phosphatase Buffer, 10x conc.	▪ 0.5 M Tris-HCl, 1 mM EDTA, pH 8.5 (+20°C). ▪ Reaction buffer for rAPid Alkaline Phosphatase.	04 898 117 001	1 vial, 1 ml
			04 898 125 001	1 vial, 1 ml
2	Rapid DNA Dephos & Ligation Kit, rAPid Alkaline Phosphatase	▪ 1 U/µl ▪ For dephosphorylation of 5′ ends. ▪ Contains no animal-derived components.	04 898 117 001	1 vial, 40 µl
			04 898 125 001	4 vials, 40 µl each
3	Rapid DNA Dephos & Ligation Kit, DNA Dilution Buffer, 5x conc.	For dilution of DNA.	04 898 117 001	1 vial, 0.5 ml
			04 898 125 001	1 vial, 0.5 ml
4	Rapid DNA Dephos & Ligation Kit, DNA Ligation Buffer, 2x conc.	Reaction buffer for T4 DNA Ligase.	04 898 117 001	1 vial, 0.5 ml
			04 898 125 001	4 vials, 0.5 ml each
5	Rapid DNA Dephos & Ligation Kit, T4 DNA Ligase	▪ 5 U/µl ▪ For ligation of dsDNA ends. ▪ 5 U/50 ng DNA standard enzyme concentration.	04 898 117 001	1 vial, 40 µl
			04 898 125 001	4 vials, 40 µl each

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	rAPid Alkaline Phosphatase Buffer, 10x conc.	Store at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***
2	rAPid Alkaline Phosphatase	
3	DNA Dilution Buffer, 5x conc.	
4	DNA Ligation Buffer, 2x conc.	
5	T4 DNA Ligase	

1.3. Additional Equipment and Reagent required

For dephosphorylation, ligation, and analysis

- Water, autoclaved deionized
- Adaptor (optional)
- Gel loading buffer: 1% SDS* (w/v), 50 mM EDTA, 0.02% bromophenol blue (w/v), 50% glycerol (v/v), pH 7.5

1.4. Application

The Rapid DNA Dephos & Ligation Kit enables fast and efficient dephosphorylation and ligation of sticky- or blunt-end DNA. Dephosphorylation requires 10 minutes, while ligation of DNA is performed in a 5 minute reaction. The recombinant rAPid Alkaline Phosphatase contained in the kit is the tool of choice for dephosphorylation of 5′ phosphates from nucleic acids.
Minimize the time needed to perform diverse applications, including:
- Cloning of fragments into either plasmid or phage vectors
- Linker ligation
- Recircularization of linear vector DNA
- Generation of libraries

1.5. Preparation Time

Assay Time

The assay time depends on the type of DNA ends to be dephosphorylated.
- Up to 1 µg DNA with blunt or sticky 5′-protruding ends in 10 minutes at +37°C.
- Up to 1 µg DNA with sticky 5′-recessive ends in 30 minutes at +37°C.

Inactivation of rAPid Alkaline Phosphatase by incubation for two minutes at +75°C.
Ligation is performed for five minutes at +15 to +25°C.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

DNA with either blunt or sticky ends (≤200 ng).

Control Reactions

Always use the following controls in a cloning/transformation experiment to determine the possible cause if results are not as expected.

Control	Result shows
Transformation of uncut vector DNA, such as 50 pg.	Transformation efficiency of cells per microgram of DNA >1 × 10^8.
Transformation of linearized unligated vector DNA.	Completeness of restriction enzyme cleavage <1% of the linearized and ligated vector.
Transformation of religated dephosphorylated vector.	Efficiency of dephosphorylation <1% of the linearized and ligated vector.
Transformation of linearized and recircularized vector.	Efficiency of ligation >5 × 10^6 blunt end, >1 × 10^7 sticky end.
Transformation without DNA, for example, with 1x DNA Dilution Buffer (Vial 3) alone.	Integrity of competent cells; no growth indicates that cells are not contaminated and do not contain a plasmid. *Growth on a selective medium indicates that the cells are contaminated and already contain a plasmid.*

General Considerations

Number of tests

The kit is designed for 40 or 160 dephosphorylation (1 µg DNA each) and ligation reactions depending on the pack size.
Standard assay: 50 ng linearized and dephosphorylated vector DNA and 150 ng insert DNA.

Standard dephosphorylation and ligation reaction

For optimal results, follow these guidelines.

Step	Recommendation
Purification	Prior to dephosphorylation and ligation, purify the DNA to the highest quality. Different kits can be used depending on the type of vector or insert DNA.
Storage of DNA	Do not store the DNA in buffers containing >0.1 mM EDTA.
Inactivation of rAPid Alkaline Phosphatase	Minimum 2 minutes at +75°C; do not inactivate >5 minutes.
Reaction Volume	▪ To prepare the DNA for ligation, dissolve the DNA in DNA Dilution Buffer, 5x conc. (Vial 3) to make a total volume of 10 µl (final concentration DNA Dilution Buffer, 1x). ▪ 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, other molar ratios can be used.* ▪ 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 dephosphorylation and ligation, purify the DNA to the highest quality. Different kits can be used depending on the type of vector or insert DNA.
Storage of DNA	Do not store the DNA in buffers containing >0.1 mM EDTA.
Inactivation of rAPid Alkaline Phosphatase	Minimum 2 minutes at +75°C; do not inactivate >5 minutes.
Reaction Volume	▪ To prepare the DNA for ligation, dissolve the DNA in DNA Dilution Buffer, 5x conc. (Vial 3) to make a total volume of 10 µl (final concentration DNA Dilution Buffer, 1x). ▪ 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 molar ratio of vector arms to insert DNA should be approximately 8:1, for example, 1,000 ng DNA, lambda gt 11, EcoR 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.***

2.2. Protocols

Standard dephosphorylation reaction

For a standard dephosphorylation reaction, perform the following steps.

i After restriction digestion, you can use the DNA directly in the dephosphorylation step. No additional purification steps are required.

1. Thaw all components listed below.

2. Briefly centrifuge all reagents before setting up the reaction.

3. Add the following reagents to a reaction vial:

Reagent	Volume [µl]	Final conc.
Vector DNA	X	up to 1 µg
rAPid Alkaline Phosphatase Buffer, 10x conc.	2	1x
rAPid Alkaline Phosphatase	1	1 U
Water, autoclaved deionized	add up to a final volume of 20	–
Total Volume	**20**	

– Mix thoroughly and centrifuge briefly.

4. Incubate DNA with blunt or sticky 5′-protruding ends for 10 minutes at +37°C.
   – Incubate DNA with sticky 5′-recessive ends for 30 minutes at +37°C.

5. Inactivate the rAPid Alkaline Phosphatase for 2 minutes at +75°C.

6. Use the dephosphorylation reaction mixture directly in the standard ligation reaction or store the mixture at −15 to −25°C until further use.

Standard ligation reaction

For a standard ligation reaction, perform the following steps.

1. Thaw all components listed below.

2. Briefly centrifuge all reagents before setting up the reaction.

3. Add the following reagents to a reaction vial:

Reagent	Volume [µl]	Final conc.
Vector DNA	X	50 ng
Insert DNA	X	150 ng
DNA Dilution Buffer, 5x conc.	2	1x
Water, autoclaved deionized	add up to a final volume of 10	–
Total Volume	**10**	

– Mix thoroughly.

4. Add 10 µl T4 DNA Ligation Buffer, 2x conc. (final concentration 1x).

5. Add 1 µl T4 DNA Ligase (final concentration 5 U).
   – Mix thoroughly.

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

7. Use the ligation reaction mixture directly for the transformation or store the mixture at −15 to −25°C.

Ligation reaction for insertion into phage vectors (including linker ligation)

Perform the following ligation protocol to insert DNA into phage vectors.
⚠ ***If the ligation requires an adaptor, add it in Step 5.***

1. For the dephosphorylation of vector arms, set up the following reaction:

Reagent	Volume [µl]	Final conc.
Vector DNA	X	up to 1 µg
rAPid Alkaline Phosphatase Buffer, 10x conc.	2	1x
rAPid Alkaline Phosphatase	1	1 U
Water, autoclaved deionized	add up to a final volume of 20	–
Total Volume	**20**	

– Mix thoroughly.

2. Incubate 10 minutes at +37°C in a water bath.

3. Inactivate the rAPid Alkaline Phosphatase for 2 minutes at +75°C.

4. Use the dephosphorylation mixture directly for the ligation reaction or store the mixture at −15 to −25°C.

5 For the ligation, set up the following reaction:

Reagent	Volume [µl]	Final conc.
Vector arms DNA, dephosphorylated	X	1,000 ng
Insert DNA	X	120 ng
Adaptor (optional)	X	–
DNA Dilution Buffer, 5x conc.	2	1x
Water, autoclaved deionized	add up to a final volume of 10	–
Total Volume	**10**	

– Mix thoroughly.

6 Add 10 µl T4 DNA Ligation Buffer, 2x conc. (final concentration 1x).

7 Add 1 µl T4 DNA Ligase (final concentration 5 U).
– Mix thoroughly.

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

9 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.

2.3. Parameters

Inactivation

rApid Alkaline Phosphatase: 2 minutes at +75°C.
T4 DNA Ligase: 10 minutes at +65°C.

Molecular Weight

rAPid Alkaline Phosphatase: 56 kD in SDS-PAGE (represents the monomer)

Specific Activity

rAPid Alkaline Phosphatase: Approximately 1 U/µg

See label for lot-specific values.

Specificity

rAPid Alkaline Phosphatase

Alkaline Phosphatase catalyzes the hydrolysis of numerous phosphate esters, such as esters of primary and secondary alcohols, saccharides, cyclic alcohols, phenols and amines.

- Phosphodiesters do not react.
- The enzyme hydrolyzes inorganic pyrophosphate.
- The kinetic properties of the enzyme depend on many factors, such as purity of enzyme, concentration of enzyme in the assay, buffer, and pH.

Temperature Optimum

rAPid Alkaline Phosphatase: +37°C

Unit Definition

One unit of rAPid Alkaline Phosphatase is the enzyme activity which hydrolyzes 1 µmol of 4-nitrophenyl phosphatase in 1 minute at +37°C under assay conditions.

Volume Activity

rAPid Alkaline Phosphatase: 1 U/µl
T4 DNA Ligase: 5 U/µl

3. Results

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% bromophenol 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 >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 pUC18 DNA, digested with either Sma I or Hind III was religated according to the standard protocol and transformed into competent *E. coli* DH5α cells. The yield of transformed colonies per µg of DNA is shown:

pUC18 DNA Undigested	pUC18/Sma I-digested (blunt or recessive ends)	pUC18/Hind III-digested (sticky ends)
1×10^8	5×10^6	1×10^7

Cloning of an insert into plasmid vectors

Fifty nanogram pUC18 DNA, digested with either Sma I or Hind III, then dephosphorylated with rAPid Alkaline Phosphatase, rec. was ligated with 150 ng either Sma I or Hind III DNA fragments. The yield of white colonies per µg of vector DNA after transformation into competent *E. coli* DH5α cells is shown:

pUC18 DNA Undigested	pUC18/Sma I (blunt end) Digest + 2,100 bp Insert DNA	pUC18/Hind III (sticky end) Digest + 2,100 bp Insert DNA
1×10^8	1×10^5	6×10^5

Cloning of an insert into phage vectors

One microgram of DNA, lambda gt 11, EcoR I arms (dephosphorylated), was ligated to 120 ng pUC18 DNA, which was linearized with either Sma I or Hind III. 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/EcoR I
5×10^7	4.9×10^4	1×10^7

4. Additional Information on this Product

4.1. Test Principle

Background information

DNA ligases join linear DNA fragments together via covalent bonds.
- DNA ligation involves creating a phosphodiester bond between the 3′ hydroxyl group of one nucleotide and the 5′ phosphate of another.
- Ligation of DNA fragments is an essential step in many molecular biology techniques, including gene cloning and messenger RNA (mRNA) fingerprinting.
- For efficient ligation, DNA strands must be prevented from self-ligating (self-circularization and concatenation) by dephosphorylation of DNA ends.
- The enzyme used to ligate DNA fragments is T4 DNA ligase, originally isolated from the T4 bacteriophage. This enzyme will ligate DNA fragments that have blunt ends or overhanging, cohesive ends that are annealed together.

Source

Recombinant alkaline phosphatase from bovine intestine expressed in *Pichia pastoris*.

4.2. Quality Control

For lot-specific certificates of analysis, see section, **Contact and Support**.

5. Supplementary Information

5.1. 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.

5.2. Changes to previous version

Layout changes.
Editorial changes.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Sodium Dodecyl Sulfate (SDS)	1 kg	11 667 289 001

5.4. Trademarks

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

5.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products and select the corresponding product catalog.

5.6. Regulatory Disclaimer

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

5.7. Safety Data Sheet

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

5.8. 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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