

RAPID DNA LIGATION KIT

(#K1421 for 10 reactions)

The Rapid DNA Ligation kit enables ligation of any type of DNA in 5 minutes at ambient temperature. The Rapid Ligation Buffer is optimized for quick and efficient ligation of cohesive-ended or blunt-ended DNA fragments [1-3].

The ligation reaction mixture can be used directly for bacterial transformation by the calcium chloride method or using the TransformAid™ Bacterial Transformation Kit (#K2711). For electrotransformation, it is necessary to inactivate T4 DNA ligase [4] by chloroform extraction.

In order to avoid self circularization and/or formation of tandem oligomers of insert and linearized vector, the optimum insert to vector molar ratio must be determined. Use the following guidelines to estimate your molar ratios:

pmol ends = pmol DNA x (number of cuts x 2 + 2),
 1µg of 1000 bp DNA = 3.04pmol ends,
 1µg of linear pUC18/19 DNA = 1.14pmol ends,
 1µg of linear pBR322 DNA = 0.7pmol ends,
 1µg of linear SV40 DNA = 0.58pmol ends,
 1µg of linear ΦX 174 DNA = 0.56pmol ends,
 1µg of linear M13mp18/19 DNA = 0.42pmol ends,
 1µg of λ phage DNA = 0.06pmol ends.

Ligation reaction mixture should contain >1- 3 fold molar excess of foreign DNA termini to vector DNA.

Recircularization of plasmid DNA can be minimized by the removal of the 5'-phosphates from both termini of cleaved vector DNA with Calf Intestinal Alkaline Phosphatase [5] or Shrimp Alkaline Phosphatase.

Another factor which may affect ligation and transformation is purity of DNA. If you chose to purify by agarose gel electrophoresis, ensure that high agarose quality is used since some preparations may contain DNA ligase inhibitors that are difficult to remove. For recovery of DNA fragments from agarose gels we recommend DNA Extraction Kit (#K0513) or Agarase (#E00461).

COMPONENTS OF THE KIT

1. T4 DNA Ligase 5u*/µl

15µl of enzyme in storage buffer containing 20mM Tris-HCl (pH 7.5), 1mM DTT, 50mM KCl, 0.1mM EDTA, 50% glycerol.

2. 5X Rapid Ligation Buffer**:

0.2ml of ready-to-use solution.

3. Water, nuclease-free:

1.5ml of 0.22µm membrane-filtered molecular biology grade water.

* One unit of enzyme catalyzes the conversion of 1 nanomole of [³²PP_i] into Norit®-adsorbable form in 20min at 37°C (Weiss unit).

** The Buffer can be thawed and frozen repeatedly. It is absolutely necessary to thoroughly mix the buffer prior to use.

METHODS

1. Insertion of Foreign DNA into Plasmid Vector

- ① To a microcentrifuge tube, add correct ratio of digested, and if preferred dephosphorylated and purified, plasmid (50-100ng)* and foreign DNA to be inserted in 5-10µl of water or TE buffer, pH 7.8**.
- ② Add the following components to the same tube:

5X Rapid Ligation Buffer	4µl
Water, nuclease-free,	up to 19µl
T4 DNA Ligase (5u)	1µl

 Vortex and spin down in a microcentrifuge for 3-5sec.
- ③ Incubate the mixture for 5 min at 22°C***.
- ④ Use 2-5µl of the ligation mixture for transformation****. The reaction mixture can be stored at 0 – 4°C until used for transformation.

* Ligation reaction mixture should contain >1-3 fold molar excess of foreign DNA termini to vector DNA. The maximum amount of vector DNA is 200ng. Ligation efficiency may decrease as insert size increases.

** Digested plasmid or DNA fragment solution can be directly used in ligation reaction if digestion was carried out in any of Fermentas Five Buffer System.

*** If a maximum yield of recombinants is essential to obtain, incubation time may be prolonged (up to 1 hour).

**** The ligation mixture can be used directly for bacterial transformation by the calcium chloride method or using the TransformAid™ Bacterial Transformation Kit (#K2711). A maximum 5µl of the ligation reaction mixture should be used for the transformation assay. For electro-transformation, it is necessary to inactivate T4 DNA ligase by chloroform extraction [4] and ethanol precipitation optional).

Typical Experiment

Blunt ended DNA fragment (approx. 2300bp length) was extracted from agarose gel using DNA Extraction Kit (#K0513) or Agarase (#E00461). The mixture containing the DNA fragments 130ng (0.17pmol ends) and dephosphorylated pUC19 DNA/SmaI digest 50ng (0.057pmol ends) was prepared and ligation reaction was performed as described above. The *E. coli* XL1-Blue competent cells, that were prepared with TransformAid™ Bacterial Transformation Kit (#K2711) in this experiment resulted in 10⁷ colonies/µg of supercoiled pUC19 DNA. Yield of white colonies after transformation into competent cells is 10⁵ colonies/µg DNA.

Analysis of ligation products by agarose gel electrophoresis

Ligation mix (10µl of the ligation product) can be analyzed by agarose gel electrophoresis by the addition of 2µl of 6X loading solution (0.09% bromophenol blue, 0.09% xylene cyanol FF, 60% glycerol, 60mM EDTA, 1% SDS). Before loading on to a gel heat the sample at 65°C for 10 min.

2. Recircularization of Linear DNA

- ① Prepare 10-35 μ l solution of linear DNA (25-50ng) in water or TE buffer, pH 7.8 in a microcentrifuge tube.
- ② Add the following components to the same tube:

5X Rapid Ligation Buffer	10 μ l
Water, nuclease-free,	up to 49 μ l
T4 DNA Ligase (5u)	1 μ l
- ③ Vortex and spin down in a microcentrifuge for 3-5sec. Incubate the tube at 22°C for 5 min.
- ④ Use 2-5 μ l of the ligation mixture for transformation****.
The reaction mixture can be stored at 0 - 4°C until used for transformation.

Typical Experiment

All the components described above were added to 30 μ l of aqueous solution containing 50ng SmaI digested pUC19 DNA. The ligation reaction was performed as described above. 1 μ l (1ng DNA) of the mixture was added to 50 μ l of *E.coli* XL1-Blue competent cells according to the procedure suggested in TransformAid™ Bacterial Transformation Kit (#K2711). Approximately 3.1x10⁶ colonies/ μ g DNA were obtained.

****The ligation mixture can be used directly for bacterial transformation by the calcium chloride method or using the TransformAid™ Bacterial Transformation Kit (#K2711). A maximum 5 μ l of the ligation reaction mixture should be used for the transformation assay. For electro-transformation, it is necessary to inactivate T4 DNA ligase by chloroform extraction [4] and ethanol precipitation (optional).

QUALITY CONTROL

Each lot of the kit is pre-tested in a ligation reaction according to the standard protocol: 130ng of blunt-ended 2300bp insert DNA was ligated to 50ng of pUC19 DNA digested with SmaI and dephosphorylated with alkaline phosphatase. Yield of white colonies after transformation into competent *E.coli* XL1-Blue cells was >1x10⁵ per μ g DNA.

Quality authorized by:

 Ramute Pineliene

TROUBLESHOOTING

Problem	Possible solution
Few or no transformants	a. Experimental DNA contains an inhibitor of ligation. Ensure DNA is free of contaminants (e.g. excess salts, EDTA, proteins, phenol, etc.) that may inhibit ligation. Gel purify and/or extract the vector and insert prior to ligation. b. Vector and/or insert have been damaged. Minimize UV exposure. c. Incompatible ends. Recheck cloning strategy. d. Incorrect vector:insert ratio. Use correct ratio. e. Cloned sequence is not tolerated in <i>E.coli</i> . If possible, check the target sequence for strong <i>E.coli</i> promoters or other potentially toxic elements, as well as inverted repeats. In case the product of a cloned gene is detrimental to a host, use promoters with a very low expression background.
High background of nonrecombinants	a. Supply LB medium with fresh ampicillin. b. Dephosphorylation of the vector helps increase ligation efficiency by eliminating recircularization of the vector. For ligation into dephosphorylated vectors, the insert must have 5'-phosphate groups.

References

1. Pfeiffer, B.H. and Zimmerman, S.B., Nucl. Acids Res., 11, 7853, 1987.
2. Hayashi, O., et al., Nucl. Acids Res. vol. 13, No 22, 7979, 1985.
3. Bercovich, J.A., et al., BioTechniques, vol. 12, No2, 190, 1992.
4. Michelsen, B.K., Anal. Biochem., 225,172, 1995.
5. Sambrook, J., and Russel D.W., Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Press, N.Y., 2001.

Trademarks

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Related Products

Calf Intestine Alkaline Phosphatase	#EF0341
	#EF0342
Shrimp Alkaline Phosphatase	#EF0511
Agarase	#E00461
Rapid DNA Ligation & Transformation Kit	#K1431
	#K1432
TransformAid™ Bacterial Transformation Kit	#K2710
	#K2711
DNA Extraction Kit	#K0513
Top Vision™ LM GQ Agarose	#R0801
Top Vision™ LE GQ Agarose	#R0491
IPTG	#R0391
	#R0392
	#R0393
X-Gal	#R0401
	#R0402
	#R0404

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Please refer to www.fermentas.com for Material Safety Data Sheet of the product.