Ready-To-Go™ T4 DNA Ligase

PRODUCT NUMBER: 27-0361-01
LOT NUMBER: 4020361011
STORE: **Ambient in pouch with desiccant**
SHIP: Ambient

Contains: A glass which when reconstituted will give a solution containing a minimum of 6 Weiss units of FPLC*pure*™ T4 DNA Ligase, 66 mM Tris-HCl (pH 7.6), 6.6 mM $MgCl_2$, 0.1 mM ATP, 0.1 mM spermidine, 10 mM DTT and stabilizers.

QUALITY CONTROL

Transformation: A minimum of 1 x 10^5 colony-forming units per µg of ligated DNA were obtained when one tube of Ready-To-Go™ T4 DNA Ligase was reconstituted with 20 µl of a solution containing 100 ng of pUC18/*Eco*R I/BAP and 100 ng of Kan^R GenBlock™ (27-4897-01) and incubated at 16°C for 30 minutes.

Blunt-end Ligation: >90% of *Sma* I fragments of Adenovirus 2 DNA were ligated within 45 minutes when 1 µg of DNA fragments in 20 µl was incubated with Ready-To-Go T4 DNA Ligase at 16°C.

Cohesive-end Ligation: >90% of *Hin*d III fragments of Lambda DNA were ligated within 30 minutes when 1 µgof DNA fragments in 20 µl was incubated with Ready-To-Go T4 DNA Ligase at 16°C.

General Protocol

To ensure that the dried ligation reaction mix is at the bottom of the tube, vigorously vortex the tube and then pulse centrifuge.

1. The DNA to be ligated can be in water, TE buffer (10 mM Tris-HCl, pH 8.0, with 1 mM EDTA), or 1-2X One-Phor-All Buffer *PLUS*. Prepare the DNA using the molar ratios specified by your standard ligation protocol. The only limitation is that no more than 20 µl can be added to a tube of Ready-To-Go T4 DNA Ligase.

2. Add 20 µl of the DNA solution to the tube of Ready-To-Go T4 DNA Ligase. If the volume of DNA added is less than 20 µl, add sufficient water to bring the final volume to 20 µl.

3. Incubate at room temperature for 3-5 minutes. Then, mix by gently pipetting up and down several times.

4. Centrifuge briefly to collect the contents at the bottom of the tube. The centrifugation will also remove any bubbles that were created in step 3.

5. Incubate at 16°C for 30-45 minutes. The ligation reaction can be heat inactivated by heating at 70°C for 10 minutes.

6. Use 2 µl of the ligation reaction to transform 100 µl of competent cells (see comment 2). If electroporation is used to transform cells, salt precipitate the DNA before electroporation (see comment 2).

Comments:

1. Typically, cohesive-ended DNA will be completely ligated after 30 minutes and blunt-ended DNA will be completely ligated after 45 minutes.

2. The reaction mix can be used directly to transform competent cells (100 µl). Adding more than 2 µl of the mix may reduce the efficiency of transformation. If more than 2 µl of the reaction mix must be used in a transformation, salt precipitate the DNA to remove stabilizers. A salt precipitation is performed after the ligation reaction as follows:

- Add 5-13 µl of a 5 M stock solution of NaCl to bring the ligation reaction to 1-2 M NaCl.
- Add 2 volumes of room temperature 100% ethanol.
- Vortex and place at -70°C for at least 1 hour.
- Centrifuge at 4°C for 10 minutes.
- Carefully remove the supernatant and discard.
- Wash the pellet two times with 1 ml of ice cold 70% ethanol. Wash by adding ethanol, vortexing, centrifuging, and carefully removing the supernatant. Discard the removed supernatant. If the salt concentration remains too high, add extra washes as required.
- Dry the DNA pellet under vacuum.
- Resuspend the pellet in a buffer compatible with subsequent use.

If carryover of protein is a concern during salt precipitation, phenol/chloroform extract the precipitated DNA before use.

3. The stabilizers that make Ready-To-Go T4 DNA Ligase ambient-temperature-stable may interfere with direct gel analysis of ligation reactions. If more than 5 µl of a ligation reaction are to be analyzed by gel electrophoresis, salt precipitation of the DNA may be required to remove the stabilizers before electrophoresis (see comment 2).

4. The inactivated ligation reaction often can be used directly in a restriction digest. However, for some restriction enzymes, it may be necessary to salt precipitate the DNA before resuspending in the appropriate digestion buffer (see comment 2). (For example, *Not* I, which is sensitive to reaction conditions, may produce two extra bands when used to digest DNA directly after ligation with Ready-To-Go T4 DNA Ligase.)

Typical Results continued on back.

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Typical Results

A. Circularization of Vector.

100 ng of pBR322 digested with *Sca* I was ligated using either Ready-To-Go™ T4 DNA Ligase or FPLC*pure*™ T4 DNA Ligase (27-0870-03, 04). Various amounts of the ligated plasmid were transformed into *E. coli* NM522. The results are reported as the number of transformants per µg of DNA.

Amount of DNA (ng)	Ready-To-Go T4 DNA Ligase	FPLC*pure* T4 DNA Ligase
1	1.5×10^8	1.6×10^8
5	2.2×10^7	2.3×10^7
10	1.2×10^7	1.2×10^7
20	4.2×10^6	5.1×10^7

E. coli NM522 transformed with 0.1 ng of undigested pBR322 yielded 1.5×10^8 transformants/µg of DNA.

B. Ligation of an Insert to a Vector.

11-46 ng of Kan^R GenBlock™ (27-4897-01) was ligated to 50 ng of pUC18 *Eco*R I/BAP (27-4854-01) using either Ready-To-Go™ T4 DNA Ligase or FPLC*pure*™ T4 DNA Ligase (27-0870-03, -04). Approximately 5 ng of the ligated insert/vector reaction was transformed into *E. coli* NM522. The results are reported as the number of transformants per µg of DNA.

Ratio of Vector to Insert	Ready-To-Go T4 DNA Ligase	FPLC*pure* T4 DNA Ligase
1:0.5	2.2×10^6	1.6×10^6
1:1	2.8×10^6	2.9×10^6
1:2	4.0×10^6	3.5×10^6

E. coli NM522 transformed with undigested pUC18 yielded 2.2×10^8 transformants/µg of DNA.

C. Ligation of an Insert to Lambda.

150 ng of Kan^R GenBlock™ (27-4897-01) was ligated to 1 µg of λgt11 *Eco*R I/CIP (27-3924-01) using either Ready-To-Go™ T4 DNA Ligase or FPLC*pure*™ T4 DNA Ligase (27-0870-03, -04). The ligated insert/phage reaction was packaged *in vitro*. The packaged phage was infected into *E. coli* Y1088. The results are reported as the number of plaques per µg of DNA.

Ratio of Insert to Vector	Ready-To-Go T4 DNA Ligase	FPLC*pure* T4 DNA Ligase
5:1	2.6×10^6	1.8×10^6

E. coli Y1088 infected with 0.1 ng of packaged 8gt11 yielded 3.7×10^8 plaques/µg of DNA.

D. Linker Ligation.

100 ng or 1 µg of phosphorylated *Bgl* II linker was ligated to 500 ng or 1 µg of pUC18 *Sma* I/BAP (27-4860-01) following the General Protocol. 10 µl of the ligation reaction was extracted with phenol/chloroform and precipitated with ethanol. The precipitated DNA was then dissolved in 1X One-Phor-All Buffer *PLUS* and digested with *Bgl* II.

Analysis of the ligation reaction on an agarose gel showed a ladder of ligation products after ligation. These products digested to a single band after treatment with *Bgl* II.

E. PCR* Fragment Ligation.

Since *Taq* DNA Polymerase adds a terminal A to the 3'-end of DNA fragments prepared by PCR, we recommend the use of SureClone™ Ligation Kit
(27-9300-01) for ligation of PCR fragments.

* The Polymerase Chain Reaction (PCR) is covered by patents owned by Roche Molecular Systems and Hoffmann-La Roche Ltd. A license to use the PCR process for certain research and development activities accompanies the purchase of certain reagents from licensed suppliers such as Amersham Biosciences and affiliates when used in conjunction with a authorized thermal cycler.

Safety warnings and precautions
This product should be handled only by persons trained in laboratory techniques and used in accordance with the principles of good laboratory practice. All chemicals should be considered potentially hazardous; therefore, when handling chemical reagents, it is advisable that suitable protective clothing, such as laboratory overalls, safety glasses and gloves be worn. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water.

Warning: For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

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