T4 RNA Ligase:

**Part No.** M105A  
**Size (units)** 500

**Description:** T4 RNA Ligase catalyzes the ATP-dependent ligation of single-stranded RNA or DNA onto the 5'-phosphoryl termini of single-stranded RNA or DNA (1,2). The enzyme, purified from recombinant Escherichia coli C44 (RNase I-deficient), has an apparent molecular weight of 43.5kDa.

**T4 RNA Ligase 10X Buffer (M107A):** When the T4 RNA Ligase 10X Buffer supplied with this enzyme is diluted 1:10, it has a composition of 50mM Tris (pH 7.8), 10mM MgCl₂, 5mM DTT and 1mM ATP.

**Enzyme Storage Buffer:** T4 RNA Ligase is supplied in 10mM Tris (pH 7.5), 50mM KCl, 0.1mM EDTA, 1mM DTT, 50% glycerol and 0.1% Tween® 20.

**Source:** Recombinant protein, expressed in *E. coli*.

**Unit Definition:** One unit is defined as the amount of enzyme required to catalyze the formation of 1 nanomole of 5'-[32P]rA14-20 into a phosphatase-resistant form in 30 minutes at 37°C at a 5' terminal concentration of 10µM. The reaction conditions are specified below under Activity Assay Conditions. See the unit concentration on the Product Information Label.

**Storage Conditions:** Store at −20°C. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. See the expiration date on the Product Information Label.

**Quality Control Assays**

**Contaminant Activity**

**DNase Assay:** To test for the absence of DNase activity, 50ng of radiolabeled DNA is incubated with 20 units of T4 RNA Ligase in T4 RNA Ligase 1X Buffer for 3 hours at 37°C. The minimum passing specification is ≤1% release of radiolabeled nucleotides as monitored by scintillation counting of TCA-soluble material.

**RNase Assay:** To test for the absence of RNase activity, 50ng of radiolabeled RNA is incubated with 20 units of T4 RNA Ligase in T4 RNA Ligase 1X Buffer for 3 hours at 37°C. The minimum passing specification is ≤1% release of radiolabeled nucleotides as monitored by scintillation counting of TCA-soluble material.

**Endonuclease Assay:** To test for endonuclease activity, 1µg of lambda or pGEM® DNA is incubated with 20 units of T4 RNA Ligase for 3 hours at 37°C. Following incubation, the DNA is visualized on an ethidium bromide-stained agarose gel to verify the absence of visible nicking.

**Physical Purity:** T4 RNA Ligase is determined to be ≥90% homogeneous as judged by SDS-polyacrylamide gels with Coomassie® blue staining.

**Activity Assay Conditions:** The RNA substrate (5'-[32P]rA14-20, 10µM of 5' termini) is ligated in the presence of T4 RNA Ligase for 15 minutes at 37°C. After ligation, the reaction is terminated by heating at 100°C for 2 minutes. The ligated substrate is then treated with 10 units of Calf Intestinal Alkaline Phosphatase (Cat.# M1821) for 10 minutes at 37°C. The amount of phosphatase-resistant substrate is monitored by scintillation counting of the TCA-precipitable material.

**References**

I. Standard Application

A. Ligation of Single-Stranded RNA

Reagents to Be Supplied by the User
- Nuclease-Free Water (Cat.# P1193)
- polyethylene glycol (PEG), 40%
- RNasin® Ribonuclease Inhibitor (Cat.# N2511/5 [Recombinant] or N2111/5 [Natural] or N2611/5 [RNasin® Plus])

1. Assemble the following reaction in a sterile microcentrifuge tube:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor RNA (see Note)</td>
<td>100–500ng</td>
</tr>
<tr>
<td>Acceptor RNA</td>
<td>250ng</td>
</tr>
<tr>
<td>T4 RNA Ligase 10X Buffer</td>
<td>4µl</td>
</tr>
<tr>
<td>RNasin® Ribonuclease Inhibitor (40u/µl)</td>
<td>1µl</td>
</tr>
<tr>
<td>PEG, 40%</td>
<td>20µl</td>
</tr>
<tr>
<td>T4 RNA Ligase (10u/µl)</td>
<td>1µl</td>
</tr>
<tr>
<td>Nuclease-Free Water to final volume</td>
<td>40µl</td>
</tr>
</tbody>
</table>

Note: Donor molecule (e.g., poly(A)+ RNA) must contain a 5’-phosphate group (PO₄). RNA molecules are efficiently phosphorylated by T4 Polynucleotide Kinase.

2. Incubate the reaction at 37°C for 30 minutes or 16°C overnight.

II. Additional Information

Molecular Weight: 43.5kDa.
Requirements: Mg²⁺ and ATP
Inactivation: Heat at 65°C for 15 minutes or at 95°C for 2 minutes.

III. Additional Applications
- Labeling the 3’-end of RNA with cytidine 3’-5’-[5’-32P]biphosphate (5’-32P)-pCp; (1).
- Intermolecular and intramolecular ligation of RNA and DNA molecules (2,3).
- Ligation of single-stranded oligodeoxyribonucleotides (4).
- Cloning full-length cDNAs (5–7).
- Incorporation of unnatural amino acids into proteins (8–11).

IV. References