Certificate of Analysis

T4 RNA Ligase:

Part No.	Size (units)
M105A	500

Part# 9PIM105

Revised 4/18

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* CA4 (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 $MgCl_2$, 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´-[^{32}P]r$A_{14–20}$ 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.

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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´-[^{32}P]r$A_{14–20}$, 10µM of 5´ termini) is ligated in the presence of T4 RNA Ligase 1X Buffer and 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.

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Signed by:

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Usage Information

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:

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

Note: Donor molecule (e.g., poly(A)+ RNA) must contain a 5′-phosphate group (PO_4). 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:	Mg^{2+} 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′-^{32}P]biphosphate (5′-[^{32}P]-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

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4. Tessier, D.C., Brousseau, R. and Vernet, T. (1986) Ligation of single-stranded oligodeoxyribonucleotides by T4 RNA ligase. *Anal. Biochem.* **158**, 171–8.
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