

Product Contents

T4 RNA Ligase:

Part No.	Size (units)
M105A	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* 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 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.

Part# 9PIM105

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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'-[³²P]rA₁₄₋₂₀, 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.

References

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2. England, T., Gumpert, R. and Uhlenbeck, O. (1977) Dinucleoside pyrophosphate are substrates for T4-induced RNA ligase. *Proc. Natl. Acad. Sci. USA* **74**, 4839-42.

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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 (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

1. England, T. and Uhlenbeck, O. (1978) 3'-terminal labelling of RNA with T4 RNA ligase. *Nature* **275**, 560–1.
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7. Schaefer, B.C. (1995) Revolutions in rapid amplification of cDNA ends: new strategies for polymerase chain reaction cloning of full-length cDNA ends. *Anal. Biochem.* **227**, 255–73.
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9. Noren, C.J. *et al.* (1990) In vitro suppression of an amber mutation by a chemically aminoacylated transfer RNA prepared by runoff transcription. *Nucl. Acids Res.* **18**, 83–8.
10. Cornish, V.W. *et al.* (1994) Site-specific incorporation of biophysical probes into proteins. *Proc. Natl. Acad. Sci. USA* **91**, 2910–4.
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