

# T4 RNA Ligase

Cat. Nos. LR5010 and LR5025

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## 1. Introduction

T4 RNA Ligase is an ATP-dependent ligase, active on a broad range of substrates including RNA, DNA, oligoribonucleotides, oligodeoxynucleotides, as well as numerous nucleotide derivatives.<sup>1-4</sup> The enzyme catalyzes the formation of a phosphodiester bond between a 5'-phosphoryl-terminated nucleic acid donor to a 3'-hydroxyl-terminated nucleic acid acceptor in a template-independent manner.<sup>5</sup> This property makes T4 RNA Ligase a valuable tool for RNA investigations by allowing intra- and intermolecular ligation events, 3'-end labeling of RNA, transcript end mapping, cDNA amplification from uncharacterized messages and the construction of unique RNA:DNA-containing oligonucleotides.<sup>1,6-12</sup>

T4 RNA Ligase is available in 1,000- and 2,500-Unit sizes at a concentration of 5 U/μl. The enzyme is supplied with a 10X Reaction Buffer and a 10 mM ATP Solution.

## 2. Applications

- **3'-end labeling of RNA species:**<sup>1,9,12</sup> The smallest donor molecule identified for T4 RNA Ligase is a nucleoside 3',5'-bisphosphate, (pNp).<sup>13</sup> The ligation of a 5'-[<sup>32</sup>P]-pNp to a 3'-hydroxylated acceptor RNA results in a 3'-phosphorylated molecule n+1 bases in length with <sup>32</sup>P-phosphate within the last phosphodiester bond.<sup>9</sup> Labeled RNA molecules can be used for RNA sequencing<sup>14</sup> or in hybridization experiments.<sup>12</sup> Other labels or modified nucleotides can be incorporated in a similar manner.
- **Synthesis of single-stranded oligonucleotides:** Series of small oligoribo- and oligodeoxyribonucleotides can be sequentially ligated together to construct molecules that would be difficult to produce efficiently by alternative means.<sup>15,16</sup> Unique RNA:RNA, RNA:DNA, and DNA:RNA molecules can be produced.<sup>1,4,6,8</sup> Such molecules have been used to study functional domains of RNA species,<sup>2,17</sup> or used to conveniently introduce multiple site-directed mutations into a molecule of interest.<sup>1</sup>
- **Intramolecular ligation of RNA molecules:** RNA molecules having both a 5'-phosphoryl and 3'-hydroxyl end can be circularized by an intra-molecular ligation event. Circular RNAs have been used in protein functional studies<sup>18,19</sup> and as templates for cDNA amplification reactions.<sup>11</sup>
- **5'- and 3'-end mapping of mRNA:** 5' and 3' termini of known and unknown mRNA can be readily mapped and amplified by a number of different techniques utilizing T4 RNA Ligase. The benefit over other established methods is that this methodology is applicable to any RNA species, it preserves the actual termini sequences, allowing the determination of precise ends and length of 3'-poly(A) tails. It also is a convenient means to amplify the sequences of interest by introducing a known primer binding site into the RNA.

In one method, 5'-capped RNA molecules are decapped with Tobacco Acid Pyrophosphatase (TAP), circularized with T4 RNA Ligase, and the 5'-3' termini junction is amplified using primers complementary to known internal sequences by RT-PCR for the study of both termini.<sup>11</sup> In a second method, mRNA is decapped with TAP, ligated to an oligoribonucleotide of known sequence at the 5' end of the mRNA, and used as a template for RT-PCR to amplify and map 5' termini.<sup>7,10,20</sup> In this reaction, amplification is primed from a gene specific oligo and an oligo complementary to the oligoribonucleotide ligated to the RNA. This has also been done using first strand cDNA as a ligation substrate instead of the mRNA.<sup>21</sup> A similar strategy is used to map

a 3' end of mRNA. An RNA or DNA oligonucleotide is ligated to the 3' end of mRNA followed by amplification of the 3' terminus using a gene specific oligo and an oligo complementary to the sequence ligated to the 3' end of the RNA.<sup>22,23</sup>

- **Synthesis of cDNA:**<sup>24</sup> The mRNA end-mapping techniques described above also define systems for the synthesis of cDNA from total cellular RNA pools.

### 3. Product Specifications

**Storage:** Store only at -20°C in a freezer without a defrost cycle.

**Storage Buffer:** T4 RNA Ligase is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 0.1 mM EDTA, 1 mM dithiothreitol (DTT), and 0.1% Triton® X-100.

**Unit Definition:** One unit catalyzes the conversion of 1 nmole of 5'-phosphoryl termini in poly-prA<sub>12-18</sub> to a phosphatase resistant form in 30 minutes at 37°C.

**Activity Assay:** The unit definition assay is performed in a reaction containing: 33 mM Tris acetate (pH 7.5), 66 mM potassium acetate, 10 mM magnesium acetate, 0.5 mM DTT, 100 µM ATP, 1 µg poly-prA<sub>12-18</sub>, and varying amounts of enzyme.

**10X Reaction Buffer:** 330 mM Tris acetate (pH 7.5), 660 mM potassium acetate, 100 mM magnesium acetate, and 5 mM DTT.

ATP is not included in the Reaction Buffer. A 10 mM solution is provided as a separate stock. ATP should be added to the reaction to a final concentration of 1 mM in 1X Reaction Buffer for intramolecular RNA ligations.

***Different reaction buffers and concentrations of ATP and enzyme are required for other applications. See specific references for the proper reaction components to use.***

**Contaminating Activity Assays:** T4 RNA Ligase is free of detectable RNase, exo- and endonuclease, and phosphatase activities.

**Enzyme Inactivation:** T4 RNA Ligase can be heat inactivated by incubation at 65°C for 10 minutes or at 95°C for 2 minutes. The enzyme can also be inactivated via removal with organic extraction (e.g., phenol/chloroform).

### 4. Related Products

The following products are also available:

- Tobacco Acid Pyrophosphatase
- MMLV-Reverse Transcriptase
- MasterAmp™ High Fidelity RT-PCR Kit
- MasterAmp™ RT-PCR Kit for High Sensitivity
- MasterAmp™ *Tth* DNA Polymerase
- APex™ Heat-Labile Alkaline Phosphatase
- T4 Polynucleotide Kinase
- RNase-Free DNase I
- T4 DNA Ligase
- Ampligase™ Thermostable DNA Ligase
- Thermostable RNA Ligase
- RNA 5' Polyphosphatase

## 5. References

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