

T4 RNA Ligase

(Cloned)

Catalog Number AM2140, AM2141

Pub. No. 4393896 Rev. B

Contents	Cat. no.	Quantity	Storage conditions
T4 RNA Ligase, 5 U/μL	AM2140	500 Units	Store at -20°C. Do not store in a frost-free freezer.
	AM2141	2500 Units	
10X T4 RNA Ligase Buffer	All sizes	1 mL	
• 500 mM Tris-HCl pH 7.8			
• 100 mM MgCl ₂			
• 100 mM DTT			
• 10 mM ATP			



WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Product description

T4 RNA Ligase catalyzes the ligation of the 5'-phosphate termini of a nucleic acid donor to the 3'-OH termini of a nucleic acid acceptor. Substrates include RNA, DNA, and oligonucleotides. The reaction is ATP dependent.

Source: an E. coli strain overexpressing T4 RNA Ligase.

Unit definition: One unit (U) is the amount of T4 RNA Ligase required to catalyze the formation of 1 nmol of 5'-[32 P]-rA₁₂₋₁₈ into a phosphatase-resistant form in 30 min at 37°C. Assay conditions: 50 mM Tris-HCl (pH 7.8), 10 mM MgCl₂, 5 mM DTT, 10 μ M 5'P-rA₁₇, 1 mM ATP.

Storage buffer (*not included***):** 20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM EDTA, 1 mM DTT, 50% glycerol.

Using T4 RNA Ligase

T4 RNA Ligase is used in the FirstChoice® RLM-RACE Kit (Cat. no. AM1700) for tagging the 5' ends of mRNA with an oligonucleotide adaptor. It can also be used in 3'-end labeling of RNA molecules, and for circularizing RNA and DNA molecules.

Procedure for 3' end-labeling of RNA (20 µL reaction):

1. Combine the following reagents in the indicated amounts:

Reagent		
2 μL 10X T4 RNA Ligase Buffer		
50-100 pmol RNA		
50–100 pmol [³² P]pCp (equimolar)		
250–500 pmol ATP (5X molar excess)		
2 μL T4 RNA Ligase (10 U)		
Nuclease-free water to 20 μL		

- 2. Mix well and incubate at 4°C for 16–96 h.
- 3. Purify the [³²P]RNA by gel electrophoresis or by size-exclusion chromatography on RNase-free Sephadex G-25 or G-50 spin columns or NucAway™ Spin Columns (Cat. No. AM10070).

Normally 10% of the radiolabel should be incorporated in the 3' end-labeling reaction.

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