epicentre®
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CircLigase™ RNA Ligase

Cat. No. TRL8101K

This enzyme was formerly called "Thermostable RNA Ligase"

1. Introduction

CircLigase RNA Ligase is a thermostable ATP-dependent ligase that catalyzes circularization (intramolecular ligation) of single-stranded RNA (ssRNA) molecules that have a 5′-phosphate and a 3′-hydroxyl end.

CircLigase RNA Ligase is provided in a 1,000-Unit size at a concentration of 100 U/µl. The enzyme is supplied with a 10X Reaction Buffer, 1-mM ATP Solution, and RNase-Free Water.

Applications

- Circularize ssRNA
- Ligation of ssRNA molecules
- Ligation of adapters to ssRNA or ssDNA
- 5′-ligation tagging of RNA molecules

2. Kit Contents

Desc.	Concentration	Quantity
CircLigase RNA Ligase		
CircLigase RNA Ligase	(100 U/µl) 1,000 Units	10 µl
10X Reaction Buffer		50 µl
1 mM ATP		20 µl
RNase-Free Water		500 µl

3. Product Specifications

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

Storage Buffer: CircLigase 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 10 pmol of a linear 5′-phosphorylated poly(A)$_{18}$ to a phosphatase-resistant form in 1 hour at 60°C under standard assay conditions.

CircLigase RNA Ligase 10X Reaction Buffer: 0.5 M MOPS (pH 7.5), 0.1 M KCl, 50 mM $MgCl_2$, and 10 mM DTT.

Note: *ATP must be added to the reaction to a final concentration of 50 µM.*

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

4. Related Products

The following products are also available:

- T4 RNA Ligase 2, Deletion Mutant
- RNA 5′ Polyphosphatase
- RiboGuard™ RNase Inhibitor
- RNase R

5. General Considerations

1. **Template Requirements:** CircLigase RNA Ligase reaction requires RNA with a 5′-phosphate and 3′-hydroxyl end. The standard reaction uses 10 pmol of RNA.
2. **Reaction Temperature:** CircLigase RNA Ligase has optimal activity at 60°C.
3. **Amount of CircLigase RNA Ligase in the Reaction:** The efficiency of the ligation reaction is strongly influenced by the sequence of the RNA. Some RNAs may be efficiently ligated using as little as 100 U of ligase, while others may require more enzyme. The standard reaction conditions presented below use 100 U of the enzyme. Therefore, you may wish to test different amounts of ligase in the reaction to determine the most effective amount of the enzyme for your RNA.

CircLigase RNA Ligase Protocol

Note: *The following protocol is for RNA circularization only; reaction conditions may differ for other applications.*

1. Thaw and thoroughly mix all reagents prior to use.
2. Combine the following components:

x	µl	RNase-Free Water
2	µl	CircLigase RNA Ligase 10X Reaction Buffer
1	µl	1 mM ATP
0.5	µl	RiboGuard RNase Inhibitor (optional; available from Epicentre)
x	µl	RNA sample (10 pmol)
1	µl	CircLigase RNA Ligase (100 Units)
20	µl	Total reaction volume

3. Gently but thoroughly mix the reaction.
4. Incubate at 60°C for 1 hour.
5. Purify the RNA by a method appropriate to the downstream application.

RiboGuard is a trademark of Epicentre, Madison, Wisconsin.

Triton is a registered trademark of Rohm & Haas, Philadelphia, Pennsylvania.