T4 RNA ligase 2

Cat.No.:HYJ237

Product Description

T4 RNA Ligase 2, also known as T4 RNA Ligase 2, is an ATP-dependent double-stranded RNA ligase (dsRNA Ligase), capable of performing both intramolecular circular ligation and intermolecular linear ligation of double-stranded RNA. Unlike T4 RNA Ligase 1, T4 RNA Ligase 2 exhibits significantly greater ligation activity towards nicks within double-stranded RNA than towards the ends of single-stranded RNA. T4 RNA Ligase 2 can also be used to connect the 3' hydroxyl group of an RNA strand to the 5' phosphate group of a DNA strand, either intramolecularly or intermolecularly within double-stranded nucleic acids (RNA duplexes, RNA/DNA hybrids, or DNA duplexes). T4 RNA Ligase 2 requires both a 5' phosphate and a 3' hydroxyl group, enabling the ligation reaction between the 5' phosphate group of an RNA or DNA strand and the 3' hydroxyl group of an RNA strand. It is primarily used to connect nicks in double-stranded RNA (Double-stranded RNA sticky-end ligation). It can also be used to connect nicks in double-stranded structures, such as the 3' hydroxyl group of RNA and the 5' phosphate group of DNA. T4 RNA Ligase 2 is a recombinant protein expressed in Escherichia coli. The gene expressed is derived from the T4 bacteriophage.

Components

Components	1KU	5KU
T4 RNA Ligase 2 (10U/μL)	100 uL	500 uL
10× Reaction Buffer	300 uL	1.5 mL
RNase-Free ddH_2O	1 mL	5 mL

Storage

Store at -25 to -15°C. Valid for 1 years.

Storage buffer

10mM Tris-HCl(pH7.5), 50mM KCl, 35mM$(NH_4)_2SO_4$, 0.1mM EDTA, 0.1mM DTT , 50% (v/v) Glycerol.

Activity Definition

One unit is defined as the amount of enzyme required to ligate 0.4 μg of an equimolar mixture of 23-mer and 17-mer RNAs in 20 μL at 37°C in 30 minutes.

Quality Control

1. Free of RNase and DNase contamination.
2. T4 RNA Ligase 2 purity >90% as determined by Coomassie Brilliant Blue-stained SDS-polyacrylamide gel.

Reaction system and conditions

1. Substrate preparation for double-stranded RNA or DNA/RNA hybrids: Incubate the RNA mixture or a mixture of DNA and RNA (at a 1:1 molar ratio) at a recommended final concentration of 20μM (10–50μM is acceptable) at 65°C for 3 minutes, followed by 2 minutes on ice.
2. Reaction system for ligating RNA 3' hydroxyl groups to DNA 5' phosphate groups in a double-stranded structure (prepared on ice):

Components	Volume
Nicked DNA/RNA Substrate	X μL
10× Reaction Buffer	2 uL
50% PEG8000	4 μL
$MgCl_2$ (100mM)	1.6 μL
T4 RNA Ligase 2(10U/μL)	1 μL
RNase-Free ddH_2O	Up to 20 uL

3. For nick ligation of double-stranded RNA, use the T4 RNA Ligase 2 ligation reaction system (prepared on ice):

Components	Volume
Nicked DNA/RNA Substrate	X μL
10× Reaction Buffer	2 uL
T4 RNA Ligase 2(10U/μL)	1 μL
RNase-Free ddH_2O	Up to 20 uL

Ligation Reaction: Incubate at 37°C for 30 minutes. If the reaction is not satisfactory, try incubating at 25°C for 2 hours. To ensure a more complete ligation reaction, the reaction time can be extended.
Termination*: Add Proteinase K or EDTA to terminate the reaction.
*Because heat inactivation of T4 RNA Ligase 2 requires heating at 85°C for 5 minutes, this may denature dsRNA or DNA/RNA hybrids. Therefore, heat inactivation of T4 RNA Ligase 2 is generally not recommended. If subsequent double-stranded DNA is not required, heating at 85°C for 5 minutes is recommended to inactivate T4 RNA Ligase 2.

Notes

1. Since RNA manipulation is involved, strict adherence to RNA handling protocols is essential to avoid RNase contamination. Reagents and consumables must be DEPC-treated to remove RNases or ensure they are RNase-free. This reaction system involves double-stranded RNA, which is resistant to RNase A and RNase T1. If single-stranded RNA is involved, including when some RNA sequences are single-stranded after dsRNA preparation, consider adding an appropriate amount of RNase inhibitor.
2. If performing multiple ligation reactions simultaneously, all solutions and enzymes listed in the table above, except for the Nicked dsRNA Substrate, can be premixed and aliquoted into individual reaction tubes. The appropriate protocol should be selected based on the specific application. Additional reagents, such as an RNase inhibitor and DEPC-free water, may be required.
3. The final concentration of the Nicked dsRNA Substrate in the reaction system can reach 1μM, which ensures sufficient ligation in this reaction system. In actual use, for example, due to limited substrate availability, the dosage of Nicked dsRNA Substrate can be appropriately reduced, for example, to a final concentration of 0.5μM or 0.2μM.
4. This product is intended for professional scientific research use only. For your safety and well-being, please wear a lab coat and disposable gloves when handling.