

T4 RNA Ligase 2

Cat. No.: M050

01/ Product Description

T4 RNA Ligase 2 is an ATP-dependent double-strand RNA ligase (dsRNA ligase). It can be used for intramolecular cyclic ligation and intermolecular linear ligation of double-stranded RNA, and can also be used for intramolecular or intermolecular RNA ligation of double-stranded nucleic acids (RNA duplex, RNA/DNA hybrid strand, or DNA duplex) for the 3' hydroxyl group of the RNA strand and the 5' phosphate group of the DNA strand. T4 RNA Ligase 2 requires the presence of 5' phosphate and 3' hydroxyl groups for ligation, which can be a ligation reaction between the 5' phosphate group present in the RNA strand or DNA strand and the 3' hydroxyl group present in the RNA strand. Treatment at 65 °C for 15 min or boiling for 2 min can inactivate the enzyme.

02/ Features

- 3' OH of RNA and 5' PO₄ of DNA in double-stranded structures can be linked;
- Ligation activity for nicks in double-stranded RNA is higher than for single-stranded RNA ends.

03/ Applications

- 1) ligation reaction of nicks in double-stranded RNA;
- 2) the ligation of the 3' hydroxyl group of RNA and the 5' phosphate group of DNA in the double-stranded structure;
- 3) Double-stranded RNA undergoes intramolecular cyclization and intermolecular linear ligation.

04/ Storage

Store at -20 °C ± 5 °C to avoid repeated freeze-thaw cycles.

05/ Storage Buffer

10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 0.1 mM DTT, 35 mM (NH₄)₂SO₄, 50% Glycerol, pH7.5.

06/ Activity Definition

One unit of enzyme activity is defined as the amount of enzyme required to ligate an equimolar mixture of 0.4 µg of 23-mer and 17-mer RNA over 30 min in a total reaction volume of 20 µl at 37 °C.

07/ Product packaging

Product composition:	M050-U100 (Size: 1000U)	M050-U500 (Size: 5000U)
T4 RNA Ligase 2 (10 U/µl)	100 µl	500 µl
10 × T4 RNA Ligase 2 Reaction Buffer	500 µl	1ml × 2
50% PEG8000	500 µl	1ml × 2

08/ Precautions

- For longer and more complex oligonucleotides, incubation at 25 °C for 2 h and then overnight at 16 °C may increase yield;
- The 10 × T4 RNA Ligation 2 Reaction Buffer in the product already contains ATP and there is no need to add it again.

09/ Experimental procedure

1. Nick ligation reaction in double-stranded RNA

- 1) Preparation of Nicked dsRNA substrates.

Equal molar ratios of the RNA mixture are heated at 65 °C for 3 min and then placed on ice for 2 min. Store at -20 °C.

- 2) Ligation reactions

Component	volume
10 × T4 RNA Ligase 2 Reaction Buffer	2 µl
T4 RNA Ligase 2 (10 U/µl)	1 µl
Nicked dsRNA Substrate (10 µM)	2 µl
RNase Free Water	Up to 20 µl

Mix well and react at 25 °C for 1 h. Add proteinase K or EDTA to stop the reaction.

2. 3' OH of RNA and 5' PO4 ligation of DNA in double-stranded structure

- 1) Prepare nick-notched DNA/RNA hybridization substrates

The DNA/RNA mixture (molar ratio equal) is heated at 65 °C for 3 min and then placed on ice for 2 min. Store at -20 °C.

- 2) Nick ligation for DNA/RNA hybridization

Component	volume
10 × T4 RNA Ligase 2 Reaction Buffer	2 µl
50% PEG8000	4 µl
MgCl ₂ (100 mM)	1.6 µl
T4 RNA Ligase 2 (10 U/µl)	1 µl
Nicked DNA/RNA Substrate (10 µM)	2 µl
RNase Free Water	Up to 20 µl

Mix well and react at 25 °C for 1 h. Add proteinase K or EDTA to stop the reaction.

10/ Related products

Cat. No.	Product name	Cat. No.	Product name
M048	T4 RNA Ligase 1	E224	RNase R
E131	T7 High Yield RNA Transcription kit	E127	DNase I
E125	RNase Inhibitor	M036	Pyrophosphatase, Inorganic (yeast)