T4 RNA Ligase 2 (dsRNA Ligase)

Product description

T4 RNA Ligase 2 is an enzyme that catalyzes the formation of a phosphodiester bond between the 5'-phosphorylated terminus and the 3'-hydroxyl terminus of an RNA strand, either intramolecularly or intermolecularly, within double-stranded RNA (dsRNA), double-stranded DNA (dsDNA), or RNA/DNA hybrid molecules. Its activity is ATP-dependent. Unlike T4 RNA Ligase 1 (Cat. No. K3151), it exhibits significantly higher ligation activity toward nicks in dsRNA than toward the termini of single-stranded RNA (ssRNA).

Ligation by T4 RNA Ligase 2 requires the presence of a 5'-phosphate and a 3'-hydroxyl group. The ligation reaction can occur between the 5'-phosphate of an RNA or DNA strand and the 3'-hydroxyl of an RNA strand. It is mainly used for intramolecular circularization of dsRNA, nick ligation (i.e., sticky-end ligation of dsRNA), and nick ligation between an RNA 3'-hydroxyl and a DNA 5'-phosphate in double-stranded nucleic acid structures.

Composition and storage conditions

Components \ Size	500 U	1000 U	5000 U	Storage
T4 RNA Ligase 2 (10 U/μL)	50 μL	100 μL	500 μL	-20°C
10× T4 RL2 Reaction Buffer	100 μL	200 μL	1 mL	-20°C
PEG8000 (50%)	200 μL	400 μL	2 mL	-20°C
$MgCl_2$ (100 mM)	100 μL	200 μL	1 mL	-20°C
Shipping: Dry Ice	**Shelf life:** 12 months			

Experimental operation

1. Prepare the following reaction systems on ice according to the table:

1) Nick ligation of double-stranded RNA (dsRNA)

Total Reaction Volume	20 μL	
10× T4 RL2 Reaction Buffer	2 μL	1×
Nicked dsRNA	X μL	final 1 μM

T4 RNA Ligase 2 (10 U/μL)	1 μL	0.5 U/μL
DEPC-treated Water	to 20 μL	

2) Nick ligation of RNA/DNA hybrid duplexes

Total Reaction Volume	20 μL	
10×T4 RL2 Reaction Buffer	2 μL	1×
Nicked DNA/RNA	X μL	final 1 μM
T4 RNA Ligase 2 (10 U/μL)	1 μL	0.5 U/μL
$MgCl_2$ (100 mM)	1.6 μL	8 μM
PEG8000 (50%)	4 μL	10%
DEPC-treated Water	to 20 μL	

2. Incubate at 25°C for 60 min for the ligation reaction (If the result is not satisfactory, you may try incubating at 37°C for 1 h or at 16°C for 16 h. To achieve a more complete ligation, the reaction time can be appropriately extended).

3. Heating at 85°C for 5 min can inactivate T4 RNA Ligase 2. Alternatively, the addition of proteinase K or EDTA can inhibit its activity and terminate the reaction.

Notes

1. Strict precautions must be taken to avoid RNase contamination during all RNA-related manipulations. Ensure that relevant reagents and consumables are either treated with DEPC to eliminate RNase or are RNase-free. Double-stranded RNA is resistant to enzymes such as RNase A and RNase T1; however, appropriate addition of RNase Inhibitor may still be considered to prevent RNA degradation.
2. When performing multiple ligation reactions in parallel, all solutions and enzymes listed in the above table except the substrate may be premixed in advance and then aliquoted into individual reaction tubes.
3. The final concentration of nucleic acid substrate in the reaction system can be up to 1 μM, at which efficient ligation can be guaranteed. In practical applications, due to limited substrate availability and other factors, the substrate dosage may be appropriately reduced, for example to a final concentration of 0.5 μM or 0.2 μM.
4. This product is for research use only!

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