T4 RNA Ligase 1 (ssRNA Ligase)

Product description

T4 RNA ligase 1 is an enzyme that can catalyze the formation of phosphodiester bonds between 5'-phosphate (5'-P) termini and 3'-hydroxyl (3'-OH) termini either within or between single-stranded RNA (ssRNA), single-stranded DNA (ssDNA), or mononucleotides, and its activity depends on ATP. T4 RNA ligase 1 exhibits the highest ligation efficiency between RNA molecules, lower efficiency between DNA and RNA, and the lowest efficiency between DNA molecules, and is therefore also regarded as a single-stranded RNA ligase.

When T4 RNA ligase 1 is used for ligation between RNA molecules, the presence of a 5'-phosphate group and a 3'-hydroxyl group is required. When it is used for ligation between RNA and mononucleotides, the mononucleotide must be phosphorylated at both the 5' and 3' termini. For ligation between DNA and RNA, higher efficiency is obtained when DNA provides the 5'-phosphate group and RNA provides the 3'-hydroxyl group, whereas ligation efficiency is very low when DNA provides the 3'-hydroxyl group and RNA provides the 5'-phosphate group. When used for ligation between DNA molecules, the efficiency is extremely low. T4 RNA ligase 1 is commonly used for RNA circularization, 3'-end labeling of RNA, and the synthesis of oligo RNA and oligo DNA.

Composition and storage conditions

Components \ Size	500 U	1000 U	5000 U	Storage
T4 RNA Ligase 1 (10 U/μL)	50 μL	100 μL	500 μL	-20°C
10× T4 RL1 Reaction Buffer	100 μL	200 μL	1 mL	-20°C
ATP (10 mM)	50 μL	100 μL	500 μL	-20°C
PEG8000 (50%, RNase free)	250 μL	500 μL	2.5 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) The circularization reaction of single-stranded RNA:

Total Reaction Volume	20 μL	
10× T4 RL1 Reaction Buffer	2 μL	1×
ssRNA	X μL	final 0.5 μM
T4 RNA Ligase 1 (10 U/μL)	1 μL	0.5 U/μL
ATP (1 mM)	1 μL	50 μM
Nuclease-free Water	Up to 20 μL	

2) The ligation reaction between single-stranded RNA or DNA

Total Reaction Volume	20 μL	
10× T4 RL1 Reaction Buffer	2 μL	1×
ssRNA/ssDNA	X μL	final 0.5 μM
T4 RNA Ligase 1 (10 U/μL)	1 μL	0.5 U/μL
ATP (10 mM)	1 μL	500 μM
PEG8000 (50%)	6-10 μL	15-25%
Nuclease-free Water	Up to 20 μL	

2. Incubate at 37°C for 30 min for the ligation reaction (If the result is not satisfactory, you may try incubating at 25°C for 2 h or at 16°C for 16 h. To achieve a more complete ligation, the reaction time can be appropriately extended).

3. Terminate the reaction by incubating at 65°C for 15 min or boiling for 2 min.

Notes

1. When working with RNA, RNase contamination must be strictly avoided. Ensure that all relevant reagents and consumables are DEPC-treated to remove RNase or are RNase-free. For single-stranded RNA reaction systems, an appropriate amount of RNase inhibitor(Cat. No. K1046) can be added to prevent RNA degradation.

2. Whether the substrate is single-stranded RNA or single-stranded DNA, it must have a phosphorylated or adenylated 5' end, and a hydroxyl group at the 3' end.

3. For ligation between nucleic acid molecules, the 3'-hydroxyl group of the nucleic acid providing the 5' phosphate is usually required to be blocked (e.g., amino modification), and the 5' end of the nucleic acid providing the 3' hydroxyl group should be blocked (e.g., 5' hydroxyl). If the amount of the nucleic acid providing the 3' hydroxyl is limited, the amount of the nucleic acid providing the 5' phosphate can be about twice that of the nucleic acid providing the 3' hydroxyl.

4. Depending on the type of ligation reaction, it is recommended to add an appropriate amount of PEG8000 to the reaction system. The final concentration of PEG8000 in the system should be 15%-25%, which can significantly enhance enzyme activity without affecting the characteristics of the reaction.

5. This product is for research use only!

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