T4 RNA Ligase 1

REF: EG25204-S/M

Storage Condition

Store at -20℃ for 2 years.

Components

Component	EG25204S	EG25204M
T4 RNA Ligase 1 (10 U/μl)	100 μl	500 μl
10× T4 Rnl1 Buffer	1.5 ml	1.5 ml
50% PEG 8000 (RNase-free)	1 ml	2×1 ml
ATP (10 mM)	200 μl	1 ml

Description

T4 RNA Ligase 1 is an ATP-dependent ligase derived from bacteriophage T4. It catalyzes the ligation of a 5' phosphoryl-terminated nucleic acid donor to a 3 hydroxyl-terminated nucleic acid acceptor through the formation of a 3'→5' phosphodiester bond with hydrolysis of ATP to AMP and PPi. The ligation efficiency of T4 RNA Ligase 1 varies significantly for different substrates, following the order: ssRNA-ssRNA ＞ ssRNA-ssDNA ＞ ssDNA-ssDNA. Additionally, T4 RNA Ligase 1 can also be used for ligation between RNA and mononucleotides, provided that the mononucleotides are phosphorylated at both the 5' and 3' ends. This application is commonly used for 3'-end labeling of RNA.

Definition of Activity Unit

One unit is defined as the amount of enzyme required to convert 1 nanomole of fluorescently labeled rA16 into a phosphatase-resistant form in 30 minutes at 37℃ .

Applications

1. RNA cyclization, RNA library construction;
2. Labeling of 3'-termini of RNA with 5'-[^{32}P] pCp;
3. Incorporation of unnatural amino acids into proteins;
4. Synthesis of Oligo RNA and Oligo DNA.

Quality Control Assays

Protein Purity

The enzyme is ≥95% pure as determined by SDS-PAGE analysis using Coomassie Blue staining.

Endonuclease Activity

A 20 μl reaction containing 200 ng of supercoiled plasmid and 10 U of T4 RNA Ligase 1 incubated for 4 hours at 37℃ results in <20% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

DNase Activity

A 20 μl reaction containing 15 ng of dsDNA fragments and 10 U of T4 RNA Ligase 1 incubated for 16 hours at 37℃ results in no detectable degradation of the dsDNA fragments as determined by agarose gel electrophoresis.

RNase Activity

A 10 μl reaction containing 500 ng of RNA and 10 U of T4 RNA Ligase 1 incubated for 1 hour at 37℃ results in >90% of the substrate RNA remains intact as determined by agarose.

Protocol

1. RNA Circularization

① Prepare the following reaction mixture on ice:

Reagent	Amount
ssRNA[a]	10 pmol
10× T4 Rnl1 Buffer	2 μl
T4 RNA Ligase 1 (10 U/μl)	1 μl
ATP (Dilute to 1 mM)[b]	1 μl
RNase Inhibitor, Murine (40 U/μl) (Optional)	0.5 μl
Nuclease-Free Water	To 20 μl

a. The amount of ssRNA in the above reaction is relatively large, for samples with fewer ssRNA, less amount of ssRNA can be added in the reaction.

b. The ATP supplied with the product has a high concentration for cyclization reactions and requires dilution before use.

② Incubate at 37℃ for 30 min. If the ligation efficiency is low, incubate at 25℃ for 1~2 h or at 16℃ for 16 h. The incubation time can be extended appropriately to make the ligation more adequate.

③ Heat inactivate at 65℃ for 15 min.

2. Intermolecular ligation of ssRNA and ssDNA

① Prepare the following reaction mixture on ice:

Reagent	Amount
ssRNA or ssDNA[c]	10 pmol
10× T4 Rnl1 Buffer	2 μl
T4 RNA Ligase 1 (10 U/μl)	1 μl
50% PEG 8000[d]	6~10 μl
ATP (10mM)	1 μl
RNase Inhibitor, Murine (40 U/μl) (Optional)	0.5 μl
Nuclease-Free Water	To 20 μl

c. For intermolecular ligation, the 3' hydroxyl group of the 5'-phosphorylated ssRNA donor should be blocked with amino or other modifications, and the 5' end of the 3'-hydroxylated ssRNA acceptor should also be blocked. If the amount of acceptor is insufficient, the amount of donor can be approximately twice as much as the amount of acceptor.

d. The final concentration of PEG8000 can be adjusted in the range of 15%~25% depending on the ligation effect.

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② Incubate at 37℃ for 30 min. If the ligation efficiency is low, incubate at 25℃ for 1~2 h or at 16℃ for 16 h. The incubation time can be extended appropriately to make the ligation more adequate.

③ Heat inactivate at 65℃ for 15 min.

3. 3' End Labeling of RNA

① Prepare the following reaction mixture on ice:

Reagent	Amount
ssRNA	1 μg
10× T4 Rnl1 Buffer	3 μl
T4 RNA Ligase 1 (10 U/μl)	1 μl
ATP (10mM)	3 μl
[^{32}P] pCp	Final concentration of 1 μM
DMSO	Final concentration of 10%
Nuclease-Free Water	To 30 μl

② Incubate overnight at 16℃ .

③ Heat inactivate at 65℃ for 15 min.

Notice

1. The ligation donor should be 5'-phosphorylated or adenylated and the acceptor should be 3'-hydroxylated.
2. The product is unable to ligate double-stranded DNA (dsDNA) or RNA.
3. RNase contamination must be strictly avoided. An appropriate amount of RNase Inhibitor (REF: EG20002) can be added to the reaction system to prevent RNA degradation.
4. When used for 3'-end labeling of RNA, dimethyl sulfoxide (DMSO) should be added to the reaction system to a final concentration of 10%.

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