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T4 RNA Ligase I

Catalog #TRL-BE101

Product Component	Sizes
T4 RNA Ligase I (30U/μL)	600U, 3000, 30kU
10X T4 RNA Ligase I Buffer	20μL, 100μL, 1mL
50% PEG 8000	100μL, 500μL, 5mL
ATP (10 mM)	10μL, 50μL, 500μL

Storage/Transportation Condition Store at -20°C ± 5°C for 24 months. Avoid repeated freeze/thaw cycles. Transport on dry ice.
Form Liquid
Source *E. coli* strain carrying the T4 RNA ligase 1 gene
Storage Buffer 10mM Tris-HCl, 50mM KCl, 0.1mM EDTA, 1mM DTT, 50% Glycerol, pH 7.4
10X T4 RNA Ligase I Buffer 500 mM Tris-HCl,100 mM $MgCl_2$,10 mM DTT，pH 7.5
Concentration 30U/μL
Unit Definition One unit is defined as the amount of enzyme required to convert 1 nmol of 5'-[^{32}P] rA_{16} into a phosphatase-resistant form in 30 minutes at 37°C.

Product Description
T4 RNA Ligase I is an ATP-dependent single-stranded DNA or RNA ligase, which catalyzes the formation of 3'→5' phosphodiester bonds between 5' phosphate group of ssRNA or ssDNA and 3' hydroxyl termini. Substrates include single-stranded RNA and DNA as well as dinucleoside pyrophosphates. The ligation activity is ssRNA-ssRNA > ssRNA-ssDNA > ssDNA-ssDNA.

Applications
- Circularization of RNA and DNA oligonucleotides
- 3' end labeling of RNA
- Intramolecular and intermolecular connections between ssDNA and ssRNA
- Connection of 3' termini of miRNA to 5' adapter probe in miRNA library construction
- Cloning of full-length cDNA

Recommended Protocol for Circularization of RNA
1. Prepare the following reaction mixture according to the table below:

Reagent	Quantity
ssRNA	200 ng – 1 μg
10X T4 RNA Ligase I Buffer	2 μL
ATP (10mM, 200X-500X)	0.04 – 0.1 μL
50% PEG 8000 (5X)	4 μL
Murine RNase Inhibitor (40U/μL)	0.5 – 1 μL
T4 RNA Ligase I (30U/μL)	1 μL
Nuclease-free H_2O	Up to 20 μL

2. Incubate at 25°C for 2 hours or at 16°C for 16 hours.
3. Stop the reaction at 65°C for 15 minutes or boil for 2 minutes.

Recommended Protocol for Ligation of ssRNA and RNA or DNA oligonucleotides
1. Prepare the following reaction mixture according to the table below:

Reagent	Quantity
ssRNA	1 – 20 pmol
RNA or DNA oligonucleotides	5 – 40 pmol
10X T4 RNA Ligase I Buffer	2 μL
ATP (10mM, 10X)	2 μL
50% PEG 8000 (3.3X)	6 μL
Murine RNase Inhibitor (40U/μL)	0.5 – 1 μL
T4 RNA Ligase I (30U/μL)	1 μL
Nuclease-free H_2O	Up to 20 μL

2. Incubate at 25°C for 2 hours or at 16°C for 16 hours.
3. Stop the reaction at 65°C for 15 minutes or boil for 2 minutes.

Notes
1. In the RNA circularization experiment, make sure that RNA has a 5' phosphate group and a 3' hydroxyl group.
2. In the ligation experiment, make sure that ssRNA contains a 3' hydroxyl group and RNA or DNA oligonucleotide contains a 5' phosphate group.
3. It is recommended to add T4 RNA Ligase1 last when making the reaction mixture.

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4. Please wear a lab coat and disposable gloves while operating.
5. For research use only.