T4 RNA Ligase 1 (ssRNA Ligase)

CATALOG NO.: M1218-100

AMOUNT: 1000 U (100 µl)

CONCENTRATION: 10 U/µl

PRODUCT SOURCE: Recombinant *E. coli*

FORM: Liquid

KIT COMPONENTS:

Components	Volume	Part No.
T4 RNA Ligase 1 (ssRNA Ligase)	100 µl (1000 U)	M1218-XX-1
10X T4 RNA Ligase Reaction Buffer	300 µl	M1218-XX-2
ATP (10 mM)	160 µl	M1218-XX-3
PEG 6000 (50% v/v)	160 µl	M1218-XX-4

DESCRIPTION: T4 RNA Ligase 1 (ssRNA Ligase) catalyzes the ATP-dependent formation of a 3'→5' phosphodiester bond by ligating the 5' phosphoryl-terminated nucleic acid donor to a 3' hydroxyl-terminated nucleic acid acceptor. It is active on a broad range of substrates including single-stranded RNA and DNA, oligoribonucleotides, oligodeoxynucleotides, as well as numerous nucleotide derivatives.

APPLICATIONS:
1. RNA 3'-termini labelling with cytidine 3',5'-bis [alpha-32P] phosphate
2. Intermolecular and intramolecular joining of RNA and DNA
3. Incorporation of unnatural amino acids into proteins
4. Synthesis of oligoribonucleotides and oligo deoxyribonucleotides
5. 5′- and 3′-end mapping of mRNA

UNIT DEFINITION: One unit is defined as the amount of T4 RNA Ligase 1 that converts 1 nmole of 5′-[32P]rA16 into a phosphatase-resistant form in 30 minutes at 37°C.

STORAGE BUFFER: 10 mM Tris-HCl (pH 7.5), 50 mM KCl, 0.1 mM EDTA, 1 mM DTT and 50% (v/v) Glycerol

STORAGE CONDITIONS: Store at -20°C. Avoid repeated freeze-thaw cycles of all components to retain maximum performance. All components are stable for one year from the date of shipping when stored and handled properly.

10X T4 RNA LIGASE REACTION BUFFER COMPONENTS: 500 mM Tris-HCl, 100 mM $MgCl_2$, 10 mM DTT, pH 7.5

REACTION CONDITIONS: Use 1X T4 RNA Ligase Reaction Buffer supplemented with 1 mM ATP and incubate at 37°C. *(Note: DMSO added to 10% (v/v) is required for pCp ligation).*

HEAT INACTIVATION: 65°C for 15 minutes

Protocol for 3′ End Labelling of RNA using T4 RNA Ligase 1:

1. Add the following components to a sterile tube at room temperature until addition of the ligase enzyme to avoid DMSO precipitation (DMSO addition is required - Not included).

Components	Volume	Final Concentration
10X T4 RNA Ligase Reaction Buffer	3 µl	1X
ATP (10 mM)	3 µl	1 mM
RNA	-	1 µg
DMSO (Not included)	-	10% (v/v)
[32P]pCp	-	1 µM
T4 RNA Ligase 1 (ssRNA Ligase)	1 µl	10 U
Nuclease-free H_2O	up to 30 µl	–

2. Collect all components by a brief centrifugation. Incubate the reaction at 16°C.

Protocol for RNA Circularization using T4 RNA Ligase 1

1. Add the following components to a sterile tube on ice. *Note: To increase the number of intramolecular ligations, increase the length of incubation and add PEG 6000.*

Components	Volume	Final Concentration
10X T4 RNA Ligase Reaction Buffer	2 µl	1X
ATP (10 mM)	-	20 - 50 µM
RNA	-	10 µM
RNaseOFF Ribonuclease Inhibitor (40 U/µl)	0.5 µl	20 U
PEG 6000 (50% v/v)	-	10% (v/v)
T4 RNA Ligase 1	1 µl	10 U
Nuclease-free H_2O	up to 20 µl	–

2. Collect all components by a brief centrifugation. Incubate the reaction at 25°C for 1-2 hours. (Increase incubation for longer oligos to overnight at 16°C).
3. To stop the reaction, boil for 2 minutes.

RELATED PRODUCTS:

- New T4 DNA Ligase M1247-200
- T4 DNA Ligase (5 u/µl) 9101-250
- T4 RNA Ligase 2 (dsRNA Ligase) M1219-100
- T4 RNA Ligase 2 (Truncated) M1220-100

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