T4 RNA Ligase 1  
(ssRNA Ligase)

1,000 units  10,000 U/ml  Lot: 0491403
RECOMBINANT  Store at –20°C  Exp: 3/16

Description: T4 RNA Ligase 1 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. Substrates include single-stranded RNA and DNA as well as dinucleoside di- and triphosphates (1).

Source: An E. coli strain that carries the T4 RNA Ligase 1 gene

Applications:
- Ligation of ss-RNA and DNA
- Labeling of 3’-termini of RNA with 5’-[32P] pCp (3)
- Inter- and intramolecular joining of RNA and DNA molecules (4,5)
- Synthesis of single-stranded oligodeoxyribonucleotides (6)
- Incorporation of unnatural amino acids into proteins (7)

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM DTT and 50% glycerol.

Reagents Supplied with Enzyme:
10X T4 RNA Ligase Reaction Buffer, 10 mM ATP and 50% PEG 8000.

Reaction Conditions: 1X T4 RNA Ligase Reaction Buffer, supplemented with 1 mM ATP. Incubate at 37°C.

1X T4 RNA Ligase Reaction Buffer:
- 50 mM Tris-HCl
- 10 mM MgCl₂
- 1 mM DTT
- pH 7.5 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to convert 1 nanomole of 5’-[32P] rA16 into a phosphatase-resistant form in 30 minutes at 37°C

Unit Assay Conditions: 1X T4 RNA Ligase reaction buffer, supplemented with 1 mM ATP, is mixed with the RNA substrate (10µM of 5’-[32P] rA16) and varying amounts of enzyme. Incubation is at 37°C for 15 minutes (8).

Heat Inactivation: 65°C for 15 minutes or boiling for 2 minutes.

Quality Control Assays
RNase Assay: Incubation of a 10 µl reaction containing 20 units of T4 RNA Ligase 1 with 40 ng of RNA transcript for 2 hours at 37°C resulted in no detectable degradation of the RNA as determined by gel electrophoresis.

DNA Exonuclease Activity: Incubation of 20 units of T4 RNA Ligase 1 with 1 µg of mixed single and double-stranded sonicated H DNA (100 cpm/µg) in 50 µl T4 RNA Ligase Reaction Buffer for 4 hours at 37°C released < 0.1% of the activity.

DNA Endonuclease Activity: Incubation of 20 units of T4 RNA Ligase 1 with 1 µg φX174 RF I DNA in 50 µl T4 RNA Ligase Reaction Buffer for 4 hours at 37°C resulted in no detectable degradation of DNA as determined by agarose gel electrophoresis.

Notes on Use: Addition of DMSO to 10% (v/v) is required for pCp ligation (3).

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References: