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]pCp 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]pCp) and varying amounts of enzyme. Incubation is at 37°C for 15 minutes.

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 Exonuclease Activity: Incubation of 20 units of T4 RNA Ligase 1 with 1 µg of X174 RF 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).

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

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