T4 RNA Ligase 2, truncated

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

The ligation product was measured on a denaturing 15% acrylamide gel. High concentrations of the adenylated DNA oligo are important for efficient ligation.

Unit Definition: 200 units is defined as the amount of enzyme required to give 80% ligation of a 31-mer DNA to the pre-adenylated end of a 17-mer DNA (Universal microRNA Cloning Linker (NEB #S1315)) in a total reaction volume of 20 µl in 1 hour at 25°C.

5′-FAM-AGGGCAGCCAAGATGGCGGAGCGGAAAGCAGG-3′
5′-rAppCTGTAGGCACCATCAAT–NH₂-3′

Unit Assay Conditions: 1X T4 RNA Ligase Reaction Buffer supplemented to 10% (w/v) PEG MW 8000, 20 pmol of 5′-FAM labeled RNA, and 40 pmol preadenylated DNA linker. After incubation at 25°C for 1 hour, the ligated product is detected on a 15% denaturing polyacrylamide gel.

Molecular Weight: 28,284.33 daltons
Specific Activity: 500,000 U/mg
Molarity: 14 µM
Heat Inactivation: 65°C for 20 minutes

Quality Control Assays
RNase Assay: A 10 µl reaction in T4 RNA Ligase Reaction Buffer containing 40 ng of labeled RNA and 200 units of T4 Rnl2tr is incubated at 25°C. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by polyacrylamide electrophoresis.

DNA Exonuclease Activity: Incubation of a 50 µl reaction containing 200 units of T4 Rnl2tr with 1 µg of a mixture of single and double-stranded "H. E. coli" DNA (200,000 cpm/µg) for 4 hours at 37°C released < 0.1% of the total radioactivity.

DNA Endonuclease Activity: Incubation of a 50 µl reaction containing 200 units of T4 Rnl2tr with 1 µg of a mixture of single and double-stranded "H. E. coli" DNA (200,000 cpm/µg) for 4 hours at 37°C resulted in <10% conversion to RF II as determined by agarose gel electrophoresis.

(see other side)
Phosphatase Activity: Incubation of 200 units of enzyme with 1 µg \( p \)-nitrophenyl phosphate (PNPP) in 50 µl T4 RNA Ligase Reaction Buffer for 3 hours at 37°C released less than 0.05 µmol inorganic phosphate.

References: