Ligate a pre-adenylated DNA or RNA sequence

**Source:** An *E. coli* strain that carries the truncated T4 RNA Ligase 2 gene.

**Applications:**
- Ligate a pre-adenylated DNA or RNA sequence tag to any RNA 3′-end
- Join a single stranded adenylated primer to small RNAs for cDNA library creation
- Join a single stranded adenylated primer to RNA for strand-specific cDNA library construction

Supplied in: 10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 0.1 mM DTT and 50% glycerol.

Reagents Supplied with Enzyme: 1X T4 RNA Ligase Reaction Buffer and 50% PEG 8000.

**Reaction Conditions:** 1X T4 RNA Ligase Reaction Buffer. Incubate at 25°C.

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 RNA to the pre-adenylated end of a 17-mer DNA (Universal miRNA Cloning Linker (NEB #S1315)) in a total reaction volume of 20 µl in 1 hour at 25°C.

5′-FAM-AGGUGCGUGUUUUGUCCGAAGAUGUUC-GCGAAUUA-3′
5′-rAppCTTAGGACCACATCAAT–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 X174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RF II as determined by agarose gel electrophoresis.

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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: