

User's Manual and Instructions

T4 RNA ligase 2, truncated

Catalog No. L1270010, L1270050 and L1270500

T4 RNA Ligase 2, truncated specifically ligates the pre-adenylated 5' end of DNA or RNA to the 3' end of RNA. The enzyme does not require ATP for ligation but does need the pre-adenylated substrate. The enzyme is expressed from a plasmid in *E. coli* which encodes the first 249 amino acids of the full length T4 RNA Ligase 2. This enzyme has been used for optimized linker ligation for the cloning of microRNAs. This enzyme reduces background ligation because it can only use adenylated primers.

Source *E. coli*

Concentration: 200 units/ μ L

Unit Definition One unit is defined as the amount of the enzyme required to give 50% ligation of a 17-mer adenylated oligonucleotide to a 17-mer purified control RNA template when both are annealed to a complementary 34-mer DNA strand in a reaction volume of 20 μ L in 30 minutes at 37°C.

Quality Control This enzyme has passed the quality control assays: SDS-PAGE analysis for purity, functional absence of endonuclease/nickase activities, functional absence of exonuclease activities, functional absence of protease activity.

Storage and Handling -20°C

Storage Buffer

50 mM Tris pH 7.5, 100 mM NaCl, 1 mM DTT, 50% glycerol, 0.1 mM EDTA, 0.1% TritonX-100.

Reaction Buffer (10X)

500 mM Tris pH 7.5, 100 mM MgCl₂, and 10 mM DTT

Quality Assurance: Free of endonucleases and exonucleases.

Components

1. T4 RNA ligase 2, truncated (200 units/ μ L)
2. 0.25 mL 10X reaction buffer.

Protocol

Mix the following reagents:

10 pmoles ssRNA
20 pmoles pre-adenylated DNA adapter
1x T4 RNA Ligase 2 truncated reaction buffer
200 U T4 RNA Ligase 2 truncated
20 μ l total volume

Incubate at 28°C for 60 min