

T4 RNA Ligase 2, Deletion Mutant

Cat. Nos. LR2D1132K and LR2D11310K

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1. Introduction

T4 RNA Ligase 2, Deletion Mutant, also known as T4Rnl2(1-249), is used to ligate single-stranded adenylated DNA or RNA oligonucleotides to small RNAs for cloning or next-generation RNA sequencing. The preadenylated 5' ends of DNA or RNA are ligated to the 3' ends of RNA. Unlike the full-length enzyme, T4Rnl2(1-249) is unable to adenylate the 5' end of the substrate in the presence of ATP. However, it can use a preactivated donor (App-DNA or App-RNA) and join it to the 3' end of an acceptor; thus, performing the ligation reaction in the absence of ATP prevents circularization and other undesirable bimolecular reactions.

The enzyme is active in a pH range of 6.0 to 8.0.

The enzyme is available in 2,000 and 10,000-unit sizes at a concentration of 200 U/ μ l. The enzyme is supplied with a 10X Reaction Buffer.

Applications

- Preparing cDNA libraries for small-RNA transcriptome analysis.
- Providing optimal linker ligation for miRNA cloning.

2. Product Specifications

Storage: Store only at -20°C in a freezer without a defrost cycle.

Storage Buffer: T4 RNA Ligase 2, Deletion Mutant, is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 0.1 mM EDTA, 1 mM dithiothreitol (DTT), and 0.1% Triton[®] X-100.

Unit Definition: One unit is the amount of enzyme required to give 50% ligation of a 22-mer RNA to the preadenylated end of a 17-mer DNA when both oligos are annealed to a complementary 39-mer DNA strand in 30 minutes at 37°C under standard assay conditions.

Activity Assay: The unit definition assay is performed in a reaction containing 50 mM Tris-HCl (pH 7.5), 2 mM MgCl_2 , 1 mM DTT, and 0.4 μg of an equimolar mix of the 22-mer, 17-mer, and 39-mer oligonucleotides, and varying amounts of enzyme.

T4 RNA Ligase 2, Deletion Mutant, 10X Reaction Buffer: 500 mM Tris-HCl (pH 7.5), 20 mM MgCl_2 , and 10 mM DTT

Contaminating Activity Assays: T4 RNA Ligase 2, Deletion Mutant, is free of detectable DNA exo- and endonuclease, and RNase activities.

3. Related Products

The following products are also available:

- ScriptMiner[™] Small RNA-Seq Library Preparation Kit (SinglePlex; Illumina[®]-compatible)
- ScriptMiner[™] Small RNA-Seq Library Preparation Kit (Multiplex; Illumina[®]-compatible)

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