T4 dsRNA Ligase

Catalogue number: MB42801, 150 U

Description

T4 dsRNA Ligase catalyses the ATP-dependent formation of a 3'→5' phosphodiester bond supporting intramolecular and intermolecular RNA strand joining. Unlike the activity of NZYTech T4 ssRNA Ligase (Cat. No. MB427), which ligates single-stranded RNA, T4 dsRNA Ligase joins nicks on double-stranded RNA and can also ligate the 3'OH of RNA to the 5' phosphate of DNA in a double-stranded structure.

Storage conditions

T4 dsRNA Ligase should be stored at -20 °C in a constant temperature freezer. The protein will remain stable till the expiry date if stored as specified.

Unit definition

One unit of enzyme activity is defined as the amount of enzyme required to ligate 0.4 µg of an equimolar mix of a 23-mer (3´-CCCGAAACGCACCCAAAGAUAUCp-5´) and 17-mer (5´-GGGCUUUGCGUGGGUUU-3´) RNAs in a total reaction volume of 20 µL in 30 minutes at 37 °C. The substrates anneal to form the following double-stranded RNA molecule, which is then ligated by the enzyme:

5´-GGGCUUUGCGUGGGUUUpCUAUAGAAACCCACGCAAAGCCC-3´
3´-CCCGAAACGCACCCAAAGAUAUCpUUUGGGUGCGUUUCGGG-5´

Enzyme concentration: 10 U/µL

Inactivation

T4 dsRNA Ligase is heat inactivated by incubation at 80 °C for 5 min.

System components and Reaction conditions

T4 dsRNA Ligase is provided with a dedicated and highly optimized NZYTech reaction buffer and displays an optimum temperature of 37 °C, although enzyme performs well at temperatures ranging from 16 °C – 37 °C.

Standard protocol

The following standard protocol serves as a general guideline to ligate nicked dsRNA. Previous to the assay, heat the RNA mixture (at equal molar ratio) at 65°C for 3 min and immediately chill on ice for 2 min. Preferably, the enzyme should be added last.

1. Prepare the following 20 µL reaction:

Component	Volume
Nicked dsRNA substrate (10 µM)	2 µL
T4 dsRNA Ligase reaction buffer (4x)	5 µL
T4 dsRNA Ligase	1 µL (10 U)
Nuclease-free H_2O (Cat. No. MB11101)	up to 20 µL

Note: *It may be required to titrate the enzyme or test different incubation periods for more effective results.*

2. Gently mix and pulse.
3. Incubate at 25 °C for 60 minutes.
4. If required, stop the reaction by adding EDTA to at least 15 mM final concentration.
5. To obtain a highly pure product, perform a column purification step using NZYGelpure kit (Cat. No. MB011).

Quality Control Assays

Purity

T4 dsRNA Ligase is >95% pure as judged by SDS polyacrylamide gel electrophoresis followed by BlueSafe staining (NZYTech, Cat. No. MB15201).

Nucleases assays

To test for DNase contamination, 0.2-0.3 µg of supercoiled pNZY28 plasmid DNA are incubated with 10 U of T4 dsRNA Ligase for 14-16 hours at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with 10 U of T4 dsRNA Ligase for 1 hour at 37 °C. Following incubation, the nucleic acids are visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acids.

Functional assay

T4 dsRNA Ligase is assayed to ligate 0.4 µg of an equimolar mix of a 23-mer (3´-CCCGAAACGCACCCAAAGAUAUCp-5´) and 17-mer (5´-GGGCUUUGCGUGGGUUU-3´) RNA oligos in a total reaction volume of 20 µL in 30 minutes at 37°C.

V2101

Certificate of Analysis

Test	Result
Enzyme purity	Pass
Nucleases contamination	Pass
Functional assay	Pass

Approved by:

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