T4 ssRNA Ligase

Catalogue number: MB42701, 1000 U

Description

T4 ssRNA Ligase catalyses the ATP-dependent formation of phosphodiester bonds between 5'-phosphate and 3'-hydroxyl termini of oligonucleotides, single-stranded RNA and DNA. Note that the minimal substrate for T4 ssRNA Ligase is a nucleoside 3',5'-biphosphate in intermolecular reaction and oligonucleotide of 8 bases in intramolecular reaction. The enzyme may be used for the ligation of ssRNA and DNA, 3´-termini of RNA labelling, synthesis of single-stranded oligoribo- and oligodeoxyribo-nucleotides and specific modifications of tRNAs.

Storage conditions

T4 ssRNA 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 is defined as the amount of enzyme required to ligate 50% of 0.4 µg of an equimolar mix of two single stranded 23 base RNA oligonucleotides (one 5'- phosphorylated) in 20 µL 1X T4 RNA Ligase Buffer following a 30 minutes incubation at 37°C.

Enzyme concentration: 10 U/µL

Inactivation

T4 ssRNA Ligase maybe inactivated at 65°C for 15 minutes or boiling for 5 minutes.

System components

T4 ssRNA Ligase is provided with a dedicated and highly optimized NZYtech reaction buffer supplemented with 1 mM ATP. and displays an optimum temperature of 37 °C.

Standard protocol

The following standard protocol serves as a general guideline for the ligation of an RNA or DNA oligo to the 3' end of ssRNA (containing a 3'-OH group) using T4 ssRNA Ligase. The protocol requires a 40% (w/v) solution of PEG 8000 and NZYRibonuclease Inhibitor (no DTT) (NZYtech, Cat. No. MB410), which are not provided. Addition of DMSO (10%) might be required in some conditions. Preferably the enzyme should be added last.

1. Prepare the following 20 µL reaction:

Component	Volume
Acceptor RNA	20 pmol
DNA or RNA oligo	40-200 pmol
T4 ssRNA Ligase reaction buffer (10x)	2 µL
NZYRibonuclease Inhibitor (no DTT)	0.5 µL
PEG 8000 40% (w/v)	7.5 µL
T4 ssRNA Ligase	1 µL (10 U)
Nuclease-free H_2O (Cat. No. MB11101)	up to 20 µL

Note: *The DNA or RNA donor should have a 5'-PO_4 group and must be blocked at the 3' end.*

2. Gently mix and pulse.
3. Incubate at 37 °C for 1 hour or at 16 °C for 16 hours.
4. To stop the reaction and obtain a highly pure product, perform a column purification step using NZYGelpure kit (Cat. No. MB011).

Quality Control Assays

Purity

T4 ssRNA 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 ssRNA Ligase for 14-16 hours at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with 10 U of T4 ssRNA 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 ssRNA Ligase is tested in a ligation reaction for the testing T4 ssRNA Ligase in the circularization of an RNA fragment synthetized *in vitro*. Efficacy of the re-ligation reaction is checked through inverse RT-PCR as RNA circularization allows the primer set to be in working distance.

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