T4 RNA Ligase 2

REF: EG25206-S/M

Storage Condition

Store at -20℃ for 2 years.

Components

Component	EG25206S	EG25206M
T4 RNA Ligase 2 (10 U/μl)	100 μl	500 μl
10× T4 Rnl2 Buffer	1 ml	2×1 ml

Description

T4 RNA Ligase 2 is an ATP-dependent double-stranded RNA ligase that exhibits both intermolecular and intramolecular RNA ligation activity. Compared to T4 RNA Ligase 1, it shows significantly higher activity for nicked double-stranded RNA than for single-stranded RNA end-joining, and requires adjacent 5' phosphate and 3' hydroxyl groups for ligation. In addition, this enzyme can ligate the 3' hydroxyl of RNA to the 5' phosphate of DNA in a double-stranded structure.

Definition of Activity Unit

One unit is defined as the amount of enzyme required to ligate an equimolar mixture of 0.4 μg of 23-mer and 17-mer RNA into a form within 30 minutes at 37℃ .

Heat Inactivation

Incubation at 80℃ for 5 minutes.

Quality Control Assays

Protein Purity

The enzyme is ≥95% pure as determined by SDS-PAGE analysis using Coomassie Blue staining.

Endonuclease Activity

A 20 μl T4 Rnl2 Buffer reaction containing 200 ng of supercoiled plasmid and 10 U of T4 RNA Ligase 2 incubated for 4 hours at 37 ℃ results in <10% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

DNase Activity

A 20 μl T4 Rnl2 Buffer reaction containing 15 ng of dsDNA fragments and 10 U of T4 RNA Ligase 2 incubated for 16 hours at 37℃ results in no detectable degradation of the dsDNA fragments as determined by agarose gel electrophoresis.

RNA Activity

A 10 μl T4 Rnl2 Buffer reaction containing 500 ng of total RNA and 1 μl of dsDNase incubated for 1 hour at 37℃ results in >90% of the substrate RNA remains intact as determined by agarose gel electrophoresis.

Phosphatase Activity

In a 200 μl reaction mixture containing 100 U of this enzyme and 2.5 mM p-nitrophenyl phosphate (pNPP), incubate at 37℃ for 4 h. Alkaline phosphatase activity is <0.0001 U.

Residual Host DNA

Using the third method of qPCR specified in General Chapter 3407 of ChP(2020) Volume IV, the residual *Escherichia coli* host cell DNA content of this product is below 10 copies/100 U.

Protocol

1. Ligation of Nicks in dsRNA

① RNA substrate preparation: Pre-treat the RNA substrate (nicked dsRNA) at 65 ℃ for 3 min, then place on ice for 2 min.

② Prepare the following reaction mixture on ice:

Reagent	Amount	Final Concentration
10× T4 Rnl2 Buffer	2 μl	1×
T4 RNA Ligase 2 (10 U/μl)	1 μl	0.5 U/μl
Nicked dsRNA Substrate (10 μM)	2 μl	1 μM
Nuclease-Free Water	up to 20 μl	-

③ Mix thoroughly and briefly centrifuge, then incubate at 25℃ for 1 h.

④ Stop reaction by adding Proteinase K or quenching with EDTA.

Notice

1. The supplied 10× T4 Rnl2 Buffer is suitable for ligating nicked double-stranded RNA. The final Mg^{2+} concentration in the buffer is 2 mM. For ligating nicks in RNA/DNA hybrid duplexes, the Mg^{2+} concentration may be increased (not exceeding 10 mM), or 10~15% PEG 8000 may be added.

2. To prevent RNase contamination, RNase inhibitors can be added. Wear clean gloves and a mask during operation. All consumables such as pipette tips and centrifuge tubes must be RNase-free.

3. Please operate with lab coatsand disposable gloves，for your safety.

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