T4 RNA Ligase 2, GMP Grade

REF: GMP109-S/M

Storage and Transportation Condition

Store at -20 ± 5℃ , Valid for 24 months. Transport at ≤ 0℃ .

Components

Component	GMP109S	GMP109M
T4 RNA Ligase 2, GMP Grade (10 U/μl)	100 μl	500 μl
10× T4 Rnl2 Buffer, GMP Grade	1 ml	2×1 ml

Description

T4 RNA Ligase 2, GMP Grade is an ATP-dependent double-stranded RNA ligase with both intermolecular and intramolecular RNA strand joining activity. Unlike T4 RNA Ligase 1, T4 RNA Ligase 2 exhibits significantly higher ligation activity toward nicked double-stranded RNA than toward single-stranded RNA ends. The ligation reaction requires adjacent 5' phosphate and 3' hydroxyl groups. In addition, this enzyme can ligate the 3' hydroxyl group of RNA to the 5' phosphate group of DNA within RNA/DNA hybrid duplex structures.

This product is manufactured and quality-controlled in compliance with GMP specifications, ensuring full traceability of the production process and raw materials. The entire production process does not involve the use of antibiotics or any animal-derived materials and excipients. Stringent controls are implemented for process-related impurities including host proteins, exogenous DNA, non-specific endonucleases, DNase, RNase, as well as microbial limits and bacterial endotoxins. This product meets the requirements for raw materials in fields such as vaccine and pharmaceutical production.

Definition of Activity Unit

One unit is defined as the amount of enzyme required to ligate 0.4 μg of an equimolar mix of a 23-mer and 17-mer RNAs in a total reaction volume of 20 μl in 30 minutes at 37℃ .

Heat Inactivation

80℃，5 min。

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 reaction in T4 Rnl2 Buffer, GMP Grade containing 200 ng of supercoiled plasmid and 10 U of this product 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 reaction in T4 Rnl2 Buffer, GMP Grade containing 15 ng of dsDNA fragments and 10 U of this product incubated for 16 hours at 37 ℃ results in no detectable degradation of the dsDNA fragments as determined by agarose gel electrophoresis.

RNase Activity

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

Phosphatase Activity

A 200 μl reaction containing 2.5 mM p-Nitrophenyl Phosphate (pNPP) and 100 units of T4 RNA Ligase 2，GMP Grade incubated for 4 hours at 37℃ yields <0.0001 unit of alkaline phosphatase activity.

Host Cell DNA

Using the third method of qPCR specified in General Chapter 3407 of ChP(2025) Volume IV, the residual *Escherichia coli* host cell DNA content of this product is below 10 copies/100 U.

Host Cell Protein

Using the method specified in General Chapter 3412 of ChP(2025) Volume IV, the residual *Escherichia coli* host cell protein content of this product is below 50 ppm.

Microbial Limit

Using the method specified in General Chapter 1105 of ChP(2025) Volume IV, the total aerobic microbial count of this product is below 1 cfu/ml, and the total combined yeasts/molds count is below 1 cfu/ml.

Bacterial Endotoxin

Using the first method of gel-clot specified in General Chapter 1143 of ChP(2025) Volume IV, the residual bacterial endotoxin content of this product is below 0.25 EU/KU.

Mycoplasma

Using a Mycoplasma detection kit (LAMP method) to test 10 U of this product, the result was negative.

Heavy Metals

Using the first method specified in General Chapter 0821 of ChP(2025) Volume IV, the residual heavy metals content of this product is below 10 ppm.

BestEnzymes Biotech Co., Ltd.

Add: No.17 Huaguoshan Avenue,Lianyungang City, China
Tel: +86 (518) 8558 6628 • E-mail:support@best-enzymes.com • www.best-enzymes.com

Protocol

1. Ligation of Nicks in dsRNA

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

② Prepare the following reaction mixture on ice:

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

③ Mix gently and spin down, incubate at 25℃ for 1 h.

④ After the reaction, add Proteinase K or EDTA to the products to terminate the reaction.

Notice

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

2. To prevent RNase contamination, RNase inhibitor can be added. Wear clean gloves and a mask during operation. All consumables such as pipette tips and centrifuge tubes used in the experiment shall be RNase-free.

3. For your safety and health, please wear a lab coat, disposable gloves, and a mask while conducting the experiment.

Add: No.17 Huaguoshan Avenue,Lianyungang City, China
Tel: +86 (518) 8558 6628 • E-mail:support@best-enzymes.com • www.best-enzymes.com