

mRNex® Gold T4 DNA Ligase (5 U/μL)

Cat. no. LD304001

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Product Information

Product	mRNex® Gold T4 DNA Ligase (5 U/μL)
Catalog No.	LD304001
Storage conditions	Stored at -20°C, valid for 2 year.
Size	1000 U/50000 U
Unit definition	One unit (U) of activity is defined as the amount of enzyme required to catalyze the ligation of more than 50% of the λDNA-Hind III fragments (6 μg) in a 20 μL reaction system at 16°C for 30 minutes.

2. Product Description

Gold T4 DNA Ligase, in the presence of ATP as a coenzyme, catalyzes the formation of phosphodiester bonds between the 5' phosphate end and 3' hydroxyl end of adjacent nucleotides on dsDNA with either blunt or sticky ends. It can also catalyze the ligation of RNA and single-stranded DNA (ssDNA) or RNA chains in double-stranded regions, but cannot catalyze the ligation of nucleotides in fully single-stranded sequences. This product is primarily used for RNA 3' end labeling, RNA cyclization, DNA oligonucleotide ligation, and cDNA cloning, as well as other nucleic acid operations.

3. Product Component

Cat No.	Component	Size	
		1000 U	50000 U
LD304001-A	Gold T4 DNA Ligase (5 U/μL)	200 μL	10 mL
LD304001-B	10 × Ligase Buffer	400 μL	20 mL

4. Quality Control

Nuclease Residual Activity Test: 10U of this product and 0.5μg of λDNA-Hind III are incubated at 37°C for 4 hours. There should be no change in the electrophoretic band of the DNA.

Endonuclease Residual Activity Test: 10U of this product and 0.5μg of IL23R plasmid are incubated at 37°C for 4 hours. There should be no change in the electrophoretic band of the DNA.

5. Notes

5.1. 1. It is normal for a small amount of precipitate to appear when the buffer is dissolved. Please invert and mix well before use.

5.2. 2. For your safety and health, please wear a lab coat and disposable gloves during operation.

5.3. 3. This product is for research use only!

6. Instructions for Use

6.1. Prepare the following reaction system in a sterile microcentrifuge tube.

Component	Usage amount
Vector DNA	50 - 100 ng
Insert fragment	The molar ratio of fragment to vector DNA ranging from 3:1 to 5:1
10 × Ligase Buffer	2 µL
Gold T4 DNA Ligase (5 U/µL)	1 µL
ddH ₂ O	To 20 µL

Note: When ligating blunt-ended vector and DNA fragments, the vector should be dephosphorylated first to prevent self-ligation. To enhance ligation efficiency, 2 µL of 50% PEG 4000 can be added to each 20 µL reaction system.

6.2. Incubate at 16 °C overnight.

6.3. Transformation experiment:

6.3.1. Add the ligation product to 100 µL competent cells (the ligation product should not exceed 1/10 of the competent cells), gently flick to mix, and incubate on ice for 30 minutes.

6.3.2. Heat-shock the tube at 42°C for 90 seconds (do not shake), then immediately place the tube on ice for 2-3 minutes.

6.3.3. Add 900 µL LB or SOC medium to the tube, incubate at 37°C, 150 rpm, and shake for 45 minutes. This process allows the bacteria to recover and express the resistance gene.

6.3.4. Centrifuge at 2500 g for 5 minutes, remove 900 µL of supernatant, resuspend the bacterial pellet in the remaining medium, and evenly spread the remaining bacterial suspension onto a selective agar plate using a sterile spreader. Once the liquid is absorbed by the plate, invert and incubate at 37°C overnight.

Note: If using super-competent cells (transformation efficiency > 10⁸ cfu/µg), directly plate 100-200 µL of the bacterial suspension after incubation at 37°C. The remaining suspension can be stored at 4°C and re-plated within 1 week