T4 DNA Ligase

T4 ligase

Item No. T665705 (100 U)
T665705 (500 U)
T665705 (5000 U)
Storage condition: -20℃

Product content

individual parts making up a compound	T665705 100 U	T665705 500 U	T665705 5 KU
T4 DNA Ligase 5 U/ L	20 L	100 L	1 mL
10×Ligation Buffer	150 L	750 L	5×1.5mL
50%PEG Solution	150 L	750 L	5×1.5mL

Product Introduction

T4 DNA Ligase, isolated and purified from E. coli expressing the T4 DNA Ligase gene after induced expression, catalyzes the reaction in which the 5' phosphate group and the 3' hydroxyl group of adjacent DNA strands are bound by a phosphodiester bond. The enzyme catalyzes the ligation of flat-ended or sticky-ended DNA and repairs single-stranded incisions in double-stranded DNA, RNA, and single-stranded DNA/RNA hybrids, but is inactive for single-stranded nucleotides.

Pre-experiment Preparation and Important Notes

1.The final dosage of T4 DNA Ligase should not exceed the recommended dosage, otherwise the ligation efficiency will be affected.
2, PEG can greatly improve the ligation efficiency of flat ends, we recommend adding a final concentration of 5% PEG Solution to improve the ligation efficiency of flat

ends.

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3. In order to improve the transformation efficiency, it is recommended that the amount of ligation product added should not exceed 10% of the volume of the receptor cells.

4. Since T4 DNA Ligase contains glycerol, which is sticky and easy to hang on the wall, it is recommended to centrifuge the liquid briefly to collect it at the bottom of the tube before use, and to collect it at the bottom of the tube when sampling.

Collect the liquid to the bottom of the tube, and try not to go too deep into the liquid surface when sampling to avoid sticking to the tip of the gun and causing loss.

Usage

i adhesive end joining

1. Reaction system.

individual parts making up a compound	20 μL reaction system	final concentration
linear vector DNA	X μL	20-100 ng
Insertion of DNA fragments	Y μL	Insert fragment: carrier 1:1-5:
10×Ligation Buffer	2 μL	/
T4 DNA Ligase 5 U/ μL	0.2 μL	1 U
ddH20	Replenish to 20 μL	20 μL

2. Vortex and shake, centrifuge momentarily and collect the solution from the wall to the bottom of the tube.

3. Reaction conditions: incubate at 22C for 10 minutes.

4. Instantaneous centrifugation, collect the solution on the wall to the bottom of the tube, incubate at 65C for 10 minutes or 70C for 5 minutes to inactivate T4 DNA Ligase.

5. 5pL of ligand product can be taken to heat shock transform 5uL of receptor cells or 15pL of ligand product can be taken to electroshock transform 50pL of receptor cells.

Note: If electroshock transformation is required, it is recommended to remove T4 DMA Ligase by ethanol precipitation before electroshock transformation.

ii Flat end connections

1. Reaction system:

individual parts making up a compound	20 μL reaction system	final con
linear vector DNA	X μL	20-100 ng
Insertion of DNA fragments	Y μL	Insert fr
10xLigation Buffer	2 μL	/
T4 DNA Ligase, 5 U/uL	1 μL	5 U
50%PEG Solution	2 L	5%
ddH20	Replenish to 20 μL	20 μL

2. Vortex and shake, centrifuge momentarily and collect the solution from the wall to the bottom of the tube.
3, Reaction conditions:Incubate at 22C for 1 hour.
4. Instantaneous centrifugation, collect the solution on the wall of the tube to the bottom of the tube, incubate at 65C for 10 minutes or 70C for 5 minutes to inactivate T4 DNA Ligase
5. Take 5L of ligase product to heat shock transform 50pL receptor cells or take 1-2L of ligase product to electroshock transform 50pL receptor cells.
Note: If electroshock transformation is required, it is recommended to remove T4 DNA Ligase by ethanol precipitation method and then carry out electroshock transformation.

iii Self-cyclization of linear DNA
1. Reaction system.

individual parts making up a compound	50 μL reaction system	final concentration
linear vector DNA	X μL	5-50 ng
10xLigation Buffer	5 μL	/
T4 DNA Ligase, 5 U/uL	1 μL	5 U
ddH20	Replenish to 50 μL	50 μL

2, Vortex shaking, momentary centrifugation, collect the solution on the wall of the tube to the bottom of the tube.
3, Reaction conditions:Sticky end incubated at 22C for 10 minutes:Flat end incubated at 22C for 1 hour.

4. Centrifuge momentarily, collect the solution on the wall of the tube to the bottom of the tube, incubate at 65C for 10 min or 70C for 5 min to inactivate T4 DNA Ligase

5. 5pL of ligation product can be taken to heat shock transform 5pL of receptor cells or 1-2pL of ligation product can be taken to electroshock transform 50pL of receptor cells.

Note: If electroshock transformation is required, it is recommended to remove T4 DNA Ligase by ethanol precipitation and then perform electroshock transformation.