5'App-T4 DNA Ligase

RK20510

Product Components

Components	Catalog	Size-1 20,000 U	Size -2 100,000 U
5'App-T4 DNA Ligase (200,000 U/mL)	RM20507	50 μL	250 μL
10X T4 DNA Ligase Reaction Buffer without ATP	RM20824	500 μL	500 μL
50% PEG8000	RM20133	1.25 mL	1.25 mL

Product Description

5'App-T4 DNA Ligase is a mutant of T4 DNA Ligase without adenylation function, which can specifically ligates the pre-adenylated 5′ end of DNA or RNA to the 3′ end of DNA. This enzyme does not require ATP as a cofactor for ligation, but requires a pre-adenylated substrate. This enzyme reduces background ligation (chimera formation) during NGS library construction, because it can only use 5 'preadenylation adapter to the DNA fragments.

Product Source

An *E. coli* strain that carries a plasmid encoding the engineered 5'App-T4 DNA Ligase gene.

Unit Definition

200 units is defined as the amount of enzyme required to give 50% ligation of a 0.25 μM double-stranded DNA to the pre-adenylated DNA in a 100 μL reaction system in 15 min at 20℃.

Storage Buffer

10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, pH 7.4 @ 25℃.

Storage Temperature

-20℃.

Reaction Conditions

1X T4 DNA Ligase Reaction Buffer without ATP, Incubate at 20℃.

1X T4 DNA Ligase Reaction Buffer without ATP

50 mM Tris-HCl,10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP, pH 7.5 @ 25℃.

Heat Inactivation

65℃ for 10 minutes.

Instructions

1. Set up the following reaction in a microcentrifuge tube on ice. (For 20 μL reaction system).

Components	Amount
10X T4 DNA Ligase Reaction Buffer without ATP*	5 μL
50% PEG8000 **	4 μL
DNA substrate	20 μL
App-Adapter	2 μL
5'App-T4 DNA Ligase (200,000 U/mL) ***	2 μL
ddH_2O	Up to 50 μL

*, 10X T4 DNA Ligase Reaction Buffer without ATP should be thawed and resuspended at room temperature., If there is a small amount of precipitation in the solution is normal, please wait for the solution to return to room temperature, shake and mix before use.

**, The dosage of PEG8000 can be adjusted according to the experimental needs.

***, 5'App-T4 DNA Ligase (200,000 U/mL) should be added last.

2. Gently mix the reaction by pipetting up and down and microfuge briefly.
3. For cohesive (sticky) ends, incubate between 20℃ for 15 minutes.
4. (Optional) Heat inactivate at 65℃ for 10 minutes.

Note: This step is optional, and the enzyme can be heat-inactivated if residual 5'App-T4 DNA Ligase will affect subsequent experiments.

QC Process

- Purity is above 95% detected by SDS-PAGE.
- No exonuclease, nuclease, RNase contamination.
- No residual host genomic DNA detected by PCR.