

Product Components

Components	Component	Size-1	Size -2
	Number	1250 U	6250 U
PBCV-1 DNA Ligase	RM21516	1 × 50 µL	1 × 250 µL
10×PBCV DNA Ligase Reaction Buffer	RM20808	1 ×100 µL	1 × 500 µL

Product Description

PBCV-1 DNA Ligase, also known as PBCV DNA Ligase, SplintR™ Ligase, or Chorella virus DNA Ligase, is an ATP-dependent DNA ligase that efficiently catalyzes the joining of two adjacent DNA single strands using a complementary RNA strand as a splint. It remains active between 16-37°C. Common applications include the detection of small RNAs such as microRNAs using probe-based methods, utilizing DNA probes to connect and detect specific RNAs, SNP or alternative splicing detection, and RASL-seq analysis..

Product Source

An *E. coli* strain that carries a plasmid encoding the engineered *PBCV-1 DNA Ligase* gene.

Unit Definition

One unit is defined as the amount of enzyme required to give 50% ligase of 2 pmol of a triple FAM-labeled DNA/RNA hybrid substrate in a 20 µL reaction system containing 1X PBCV DNA Ligase Reaction Buffer, incubated at 25°C for 15 minutes..

Storage Buffer

10 mM Tris-HCl, 300 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, pH 7.4 @ 25°C.

Storage Temperature

-20°C.

Reaction Conditions

1 X PBCV DNA Ligase Reaction Buffer, Incubate at 25°C.

1X T4 DNA Ligase Reaction Buffer

50mM Tris-HCl, 10mM MgCl₂, 1mM ATP, 10mM DTT, pH 7.5 @ 25°C.

Heat Inactivation

65°C for 20 minutes.

Instructions

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

Components	Amount
10X PBCV DNA Ligase Reaction Buffer*	2 µL
Substrate DNA (1 µM)	2 µL
PBCV-1 DNA Ligase ***	1 µL
ddH ₂ O	Up to 20 µL

*, 10X PBCV DNA Ligase Reaction Buffer 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.

***, PBCV-1 DNA Ligase should be added last.

2. Gently mix the reaction by pipetting up and down and microfuge briefly.

3. Ligation at 25°C for 15-60 minutes.
4. Heat inactivate at 65°C for 20 minutes.
5. Chill on ice for downstream experiments, or store at -20°C.