



ssDNA/RNA CircLigase

User's Instruction

Description

ssDNA/RNA CircLigase is a thermostable DNA/RNA ligase, which is independent of ATP. It can catalyze the cyclization of single stranded DNA or RNA, and intermolecular ligation, but the efficiency is higher when use single stranded DNA as substrate. During cyclization, 5' - phosphate group and 3' - OH group are required in the substrate single stranded DNA or RNA. For single stranded substrates larger than 15 nt, ssDNA/RNA CircLigase works efficiently. The enzyme can also catalyze the ligation between DNA or RNA molecules and pre-adenylated DNA junctions. In addition, in the case of excessive ATP, the enzyme can adenosylate DNA/RNA molecules.

Kit Contents

	2 KU
1. ssDNA/RNA CircLigase (100 U/μl)	20 μl
2. 10 × CircLigase Buffer	250 μl
3. 50mM MnCl ₂	100 μl
<ul style="list-style-type: none"> • 1×CircLigase Buffer: 30 mM Tris-acetate (pH 7.5), 60 mM potassium acetate, 1 mM DTT. 	

Unit Definition

One unit is defined as the amount of enzyme required to catalyze 1 pmol of a linear 5'-phosphorylated control oligo (55 mer) into circular ssDNA in 1 hour at 60°C.

Protocol

1. Set up the reaction as the following table:

Component	Volume
ssDNA/RNA CircLigase (100 U/μl)	1 μl



ssDNA/RNA	10 pmol
10 × CircLigase Buffer	2 μl
50mM MnCl ₂	1 μl
ddH ₂ O	Up to 25 μl

2. Thermocycling Conditions

- a) 60°C for 1 h (cyclization)
- b) 80°C for 10 min (inactivation)

3. After 20% acrylamide / 8M urea denaturation, run the gel electrophoresis. Stain the reaction products to observe the cyclization. The uncyclized or adenosylated substrates could be digested by exonuclease I.

Note

- The addition of 50 μM ATP can improve the cyclization efficiency, while the addition of ATP with concentration higher than 1 mM can inhibit the ligation.
- For the ligation of single stranded DNA products, the final concentration of 2.5 mM MnCl₂ should be added.
- MnCl₂ is not needed for the ligation of single stranded RNA products.
- For the substrates with secondary or complex structure, 0.4 ~ 0.8 M Betain can be added to improve the efficiency.
- The enzyme has selectivity for substrate base. 5' terminal ligation efficiency: G > A >> T(U) >> C, 3' terminal ligation efficiency: T(U) > A > G. When the 3' terminal base is C, it cannot be used for ligation, as there will be almost no ligation products.

Storage

Minimum shelf life is 3 year at -20°C.