Prokazyme

Product number: Dlig120
Lot number:

Rhodothermus marinus DNA ligase

Introduction

Product Description

Rma DNA ligase catalyzes the NAD-dependent ligation of adjacent 3´-hydroxyl and 5´-phosphate termini in duplex DNA structures. In contrast to T4 DNA ligase, Rma DNA ligase has no detectable activity on blunt end DNA fragments.

Unlike T4 DNA ligase, Rma DNA ligase shows only minimal ligation activity under optimal temperature conditions for 4 bp as well as 2 bp of cohesive ends. Rma DNA ligase has no activity on RNA targets.

Rma DNA ligase is isolated and purified from an *E.coli* strain carrying a plasmid with the cloned DNA ligase gene from the thermophilic bacteria *Rhodothermus marinus* isolated in Iceland (1, 2).

The half-life of Rma ligase is 7 min at 91°C (2). The enzyme has a broad range of reaction temperatures with the optimal activity about 55°C. Under optimal conditions the rate and extent of oligonucleotide ligation is higher for Rma DNA ligase compared to Tth and Taq DNA ligases (3,4).

Applications

Rma DNA ligase is an ideal enzyme for applications requiring high temperature, high-stringency ligations of double-stranded DNA.*Rma* DNA ligase may be applied to:

- Gene Synthesis (12) from overlapping oligonucleo-tides

Storage

Storage and dilution buffer: 20 mM Tris-HCl, 50 mM KCl, 0,1 mM EDTA, 0,1% Triton X-100 (v/v), 1 mM dithiothreitol (DTT), 50% glycerol (v/v), pH 7,6 (25°C). Rma DNA ligase is stable when stored at –15°C to -25°C.

Reaction Conditions for unit definition

1 x reaction buffer (10 x supplied) 20 mM Tris-HCl, 20 mM KCl, 10 mM MgCl2, 0,1% Nonidet P40 (v/v), 0,5 mM NAD, 1 mM DTT, pH 7,5 (25°C).

Concentration and Unit Definition

Concentration 10 U/µl.

One unit of *Rma* DNA Ligase catalyzes the ligation of 50% of the cos sites of 1 µg BstEII digested λDNA in 1 min at 45°C.

Application protocol

Reaction Protocol

Example of oligonucleotide ligation:

Thaw the components listed below and place them on ice. Vortex briefly and centrifuge all reagents before setting up the reactions. Set up the reaction components in a microfuge tube placed on ice:

Component	Volume	Final conc.
Reaction buffer (10x)	2,0 µl	1 x
Oligo 1	X µl	1-30nM
Oligo 2	X µl	1-30nM
Template DNA	X µl	0,1 ng
Tsc DNA Ligase	0,5 µl	5 U
Add sterile H2O	Up to 20,0 µl	
TOTAL	20,0 µl	

A typical temperature profile is: 94°C 2 min, 94°C 30 sec, 45-65°C 3 min and repeat last two temperatures for 30 cycles. 99°C for 10 min.

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Activity Assay

The enzyme assay for unit definition was ligation of cos sites of λ-DNA digested with BstII.

Component	Volume	Final conc.
Reaction buffer (10x)	2,0 µl	1 x
λ -DNA (BstEII digested)	X µl	1 µg
Rma DNA Ligase	Dilution series	
Add sterile H2O	Up to 20,0 µl	
TOTAL	20,0 µl	

Incubate at 45°C for 1-15 min. Stop reaction in dry ice/ethanol bath Incubate for 10 min at 65°C before analysis on agarose gel (melting of not ligated cos sites). Results are assayed by agarose gel electrophoresis and ethidium bromide staining

Quality Control

Each lot of *Rma* DNA Ligase is assayed for activity and for contaminating activities as stated below.

Absence of DNA endonuclease

- 0,25 µg supercoiled pBR322 DNA is incubated with increasing amounts of *Rma* DNA ligase in 25 µl reactions at 37°C for 16 h. >100 U of *Rma* DNA ligase show no relaxation of the supercoiled structure of pBR322 DNA.
- 0,25 µg of λ-DNA Eco RI/HindIII fragments is incubated with *Rma* DNA ligase in 25 µl reactions at 37°C and 64°C for 16 h. 100 U of *Rma* DNA ligase show no alteration of the banding pattern.

Absence of exonuclease

Increasing amounts of *Rma* DNA ligase are incubated in 50 µl test buffer containing [3H]-labelled DNA at 37°C and 64°C for 4 h. The amount of enzyme, which shows no exonuclease activity is >100 U.

Absence of Rnases

RNaseAlert™ Lab Test Kit (cat no. 1964) from Ambion was used to detect RNase activity according to the manufacturer protocol. No RNase activity was detected after incubating >50 U of *Rma* DNA ligase at 37°C after 1 hour.

References

1. Alfredsson GA, et al. 1988. J. Gen. Microbiol. 134:299-306
2. Torbjarnardottir SH et al. 1995. Gene 161: 1-6
3. Housby JN, et al. 2000. Nucleic Acids Research. 28: e10.
4. Housby JN, et al. 2001. Anal. Biochem. 302: 88-94
5. Sutton JR, et al. 1992. Transgenic Research 1: 228

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Safety handling – All enzymes bear the warning:

- HARMFUL ENZYME-PROTEIN
- Enzymes may cause sensitization by inhalation

Caution:

- Not for diagnostic use
- The safety and efficacy of this product in diagnostic or other clinical use has not been established

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- It is free of biological and chemical hazards
- This product is distributed for laboratory use only