**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 oligonucleotide-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:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
<th>Final conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction buffer (10x)</td>
<td>2.0 µl</td>
<td>1 x</td>
</tr>
<tr>
<td>Oligo 1</td>
<td>X µl</td>
<td>1-30nM</td>
</tr>
<tr>
<td>Oligo 2</td>
<td>X µl</td>
<td>1-30nM</td>
</tr>
<tr>
<td>Template DNA</td>
<td>X µl</td>
<td>0.1 ng</td>
</tr>
<tr>
<td>Tsc DNA Ligase</td>
<td>0.5 µl</td>
<td>5 U</td>
</tr>
<tr>
<td>Add sterile H2O</td>
<td>Up to 20.0 µl</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>20.0 µl</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
<th>Final conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction buffer (10x)</td>
<td>2.0 µl</td>
<td>1 x</td>
</tr>
<tr>
<td>λ-DNA (BstEII digested)</td>
<td>X µl</td>
<td>1 µg</td>
</tr>
<tr>
<td>Rmam DNA Ligase Dilution series</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Add sterile H2O</td>
<td>Up to 20.0 µl</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>20.0 µl</td>
<td></td>
</tr>
</tbody>
</table>

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 Rmam 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 Rmam DNA ligase in 25 µl reactions at 37°C for 16 h. >100 U of Rmam 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 Rmam DNA ligase in 25 µl reactions at 37°C and 64°C for 16 h. 100 U of Rmam DNA ligase show no alteration of the banding pattern.

Absence of exonuclease
Increasing amounts of Rmam 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 Rmam DNA ligase at 37°C after 1 hour.

References

Limited Usage Statement
The purchase of this product conveys to the buyer the non-transferable right to use the product and components of the product in research conducted by the buyer. The buyer cannot sell or otherwise transfer this product or its components to a third party and in particular, no rights are conveyed to the buyer to use the product or its components for commercial use purpose.

The information contained in this leaflet is, to the best of our knowledge, true and accurate, but since the conditions of use are beyond our control, no warranty is given or to be implied in respect of any recommendation or suggestions which may be made or that any use will not infringe any patents.

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

This product is produced by Prokazyme Ltd., Gylfaflot 5, 112 Reykjavik, Iceland.
- It is free of biological and chemical hazards
- This product is distributed for laboratory use only

Prokazyme Ltd. • Gylfaflöt 5, IS-112 Reykjavik, Iceland • www.prokazyme.com • info@prokazyme.com