

PRODUCT NUMBER: Dlig 119

LOT NUMBER: 351-0302-02



PROKARIA

# Tsc DNA ligase

## I. INTRODUCTION

### Product description

Tsc 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, Tsc DNA ligase has no detectable activity on blunt end DNA fragments. Unlike T4 DNA ligase, Tsc DNA ligase shows only minimal ligation activity under optimal temperature conditions for 4 bp as well as 2 bp of cohesive ends. Tsc DNA ligase has no activity on RNA targets. Tsc DNA ligase is isolated and purified from an *E.coli* strain carrying a plasmid with the cloned DNA ligase gene from the thermophilic bacteria *Thermus scotoductus* isolated in Iceland (1, 2). The half-life of Tsc ligase is 26 min at 91°C (3). The enzyme has a broad range of reaction temperatures with the lower limit around 15°C and the upper limit determined by the melting temperature ( $T_m$ ) of the DNA substrate. The enzyme is also active in various DNA polymerase buffers within the pH range of 7-9. Under optimal conditions the rate and extent of oligonucleotide ligation is much higher for Tsc DNA ligase compared to other commonly available thermostable ligases (4,5).

### Applications

Tsc DNA ligase is an ideal enzyme for applications requiring high temperature, high-stringency ligations of double-stranded DNA.

Tsc DNA ligase may be applied to:

- Ligase Chain Reaction (LCR) (6-8) for amplification of DNA targets
- Oligonucleotide Ligation Assay (OLA) (9-10) for mutational analysis
- Repeat Expansion Detection (11) for determining genetic anomalies, such as trinucleotide repeats, research only, not for diagnostic purposes (for e.g. Fragile X, Huntington disease)
- Gene Synthesis (12) from overlapping oligonucleotides

### Storage and stability

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). Tsc Ligase is stable when stored at -15°C to -25°C

### Reaction conditions

1 x reaction buffer (10 x supplied) 20 mM Tris-HCl, 20 mM KCl, 10 mM MgCl<sub>2</sub>, 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 Tsc DNA Ligase catalyzes the ligation of 50% of the cos sites of 1μg BstEII digested λDNA in 1 min at 45°C

## II. APPLICATION PROTOCOL

### Activity assay for unit determination

- The enzyme assay for unit definition was ligation of cos sites of λDNA digested with BstEII.

Component	Volume	Final conc.
Reaction buffer (10x)	2,0 μl	1 x
λDNA (BstEII digested)	X μl	1 μg
Tsc DNA Ligase	Dilution serie	
Add sterile H <sub>2</sub> O	Up to 20,0 μl	
<b>TOTAL</b>	<b>20,0 μl</b>	

- 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

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### 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 H <sub>2</sub> O	Up to 20,0 µl	
<b>TOTAL</b>	<b>20,0 µl</b>	

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.

### III. QUALITY CONTROL

#### Quality Control

Each lot of Tsc 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 Tsc DNA ligase in 25 µl reactions at 37°C for 16 h. >100 U of Tsc DNA ligase show no relaxation of the supercoiled structure of pBR322 DNA.

**AND**

0,25 µg of λ-DNA Eco RI/HindIII fragments is incubated with Tsc DNA ligase in 25 µl reactions at 37°C and 64°C for 16 h. 75 U of Tsc DNA ligase show no alteration of the banding pattern.

#### Absence of exonuclease

Increasing amounts of Tsc DNA ligase are incubated in 50 µl test buffer containing [<sup>3</sup>H]-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 Tsc DNA ligase at 37°C after 1 hour.

### IV. References

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**This product is produced by Prokaria Ltd. Reykjavik, Iceland.**

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

#### CAUTION

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

**Safety handling – All enzymes bear the warning:**

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

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### **LIMITED USE 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

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