Product number: Dlig119
Lot number:

Thermus scotoductus DNA ligase

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 (Tm) of the DNA substrate.

The enzyme is 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, re-search only, not for diagnostic purposes (for e.g. Fragile X, Huntington disease)
- 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). *Tsc* 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 *Tsc* 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
Tsc DNA Ligase	Dilution serie	
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 *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.
- 0,25 µg of λ-DNA Eco RI/HindIII fragments is incu-bated with *Tsc* DNA ligase in 25 □l 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 [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 *Tsc* DNA ligase at 37°C after 1 hour.

References

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