enzymatics®
the new standard in enzyme production
Biozym
SCIENCE IS OUR BUSINESS

Product Information Sheet

Product Information	
***E. coli* DNA Ligase**	
Part Number	L6090L 280200
Price	
Concentration	10,000 U/mL
Unit Size	2,500 U

Product Specification	
Storage Temperature	-25°C to -15°C
TEST:	**SPECIFICATION:**
Purity (SDS-PAGE)	>99%
Specific Activity	20,000 – 28,880 U/mg
SS Exonuclease	100 U < 2.0% released
DS Exonuclease	100 U < 1.0% released
DS Endonuclease	100 U = no conversion
***E.coli* DNA Contamination**	50 U = 0 copies

Product Description:

E. coli DNA ligase catalyzes the phosphodiester bond formation between an adjacent 5' phosphate and a 3' hydroxyl of DNA ends, requiring NAD^+ and Mg^{2+} as cofactors. Ligation of blunt ended DNA is extremely inefficient relative to cohesive DNA end ligation and nick sealing.

Source of Protein

The gene encoding *E. coli* DNA Ligase expressed from a plasmid in *E. coli*.

Supplied in

10 mM Tris-HCl
50 mM KCl
1 mM DTT
0.1 mM EDTA
50% glycerol
pH 7.5 @ 25°C

Supplied with

B6090 (10X *E.coli* DNA Ligase Reaction Buffer)

10X *E.coli* DNA Ligase Reaction Buffer (B6090):

300 mM Tris-HCl
40 mM $MgCl_2$
260 µM NAD
10 mM DTT
0.5 mg/mL BSA
pH 8.0 @ 25°C

Unit Definition

1 unit is defined as the amount of *E.coli* DNA Ligase required to ligate 50% of 100 ng DNA fragments with cohesive termini in 30 minutes at 25°C.

Quality Control Analysis:

Unit Characterization Assay

Unit activity was measured using a 2-fold serial dilution method. Dilutions of enzyme batch were made in 1X reaction buffer (30 mM Tris-HCl, 4 mM MgCl2, 1 mM DTT, 26 µM NAD, 50 µg/mL BSA, pH 8.0 @ 25°C, [*E.coli* DNA Ligase]$_f$ = 0.42-0.0033 mg/mL) and added to 25 µL reactions containing 165ng DNA and 1X reaction buffer. Reactions were incubated 30 minutes at 25ºC (room temp), plunged on ice, and analyzed on a 1% agarose gel stained with ethidium bromide. Specific activity was equivalent to reference sample.

Protein Concentration (OD_{280}) Measurement

A 2.0 µL sample of enzyme was analyzed at OD_{280} using a Nanodrop ND-2000 spectrophotometer standardized using a 2.0 mg/ml BSA sample (Pierce Cat #23209), and blanked with product storage solution. The observed average measurement of 3 replicate samples was converted to mg/mL using an extinction coefficient of 34,280 and molecular weight of 73,606 Daltons.

SDS-Page (Physical Purity Assessment) and Specifications

2.0 µL of enzyme solution was loaded on a denaturing 4-20% Tris-Glycine SDS-PAGE gel flanked by a broad-range MW marker and 2.0 µL of a 1:100 dilution of the sample. Following electrophoresis, the gel was stained and the samples compared to determine physical purity. The acceptance criteria for this test requires that the

aggregate mass of contaminant bands in the concentrated sample do not exceed the mass of the protein of interest band in the dilute sample, confirming greater than 99% purity of the concentrated sample.

Contamination Tests:

Single-Stranded Exonuclease Activity

A 50 µL reaction containing 10,000 cpm of a radiolabeled single-stranded DNA substrate and 10 µl of enzyme solution incubated for 4 hours at 37°C resulted in less than 2.0% release of TCA-soluble counts.

Double-Stranded Exonuclease Activity

A 50 µL reaction containing 5,000 cpm of a radiolabeled double-stranded DNA substrate 10 µl and of enzyme solution incubated for 4 hours at 37°C resulted in less than 1.0% release of TCA-soluble counts.

Double-Stranded Endonuclease Activity

A 50 µL reaction containing 0.5 µg of pBR322 DNA and 100 Units of enzyme solution incubated for 4 hours at 37°C resulted in no visually discernible conversion to nicked circular DNA as determined by agarose gel electrophoresis.

E.coli 16S rDNA Contamination Test

Replicate 15,000 Unit samples of enzyme solution were denatured and screened in a TaqMan qPCR assay for the presence of contaminating *E.coli* genomic DNA using oligonucleotide primers corresponding to the 16S rRNA locus. The acceptance criterion for the test is the threshold cycle count (C_t) produced by the average of 3 replicate no template control samples. Based on the correlation between the no template control C_t values, and standard curve data, the detection limit of this assay is <10 copies genome/sample.

References:

1. Lehman I.R. (1974) Science 186, 790-797.

Biozym
SCIENCE IS OUR BUSINESS

Biozym Scientific GmbH
Steinbrinksweg 27
D-31840 Hessisch Oldendorf
Tel.: +49 (0)5152-9020
Fax: +49 (0)5152-2070
support@biozym.com
www.biozym.com

enzymatics®
the new standard in enzyme production

Limitations of Use

This product was developed, manufactured, and sold for *in vitro* use only. The product is not suitable for administration to humans or animals. MSDS sheets relevant to this product are available upon request.