



Product Information

Taq DNA Ligase

Part Number:	L606L
Lot Number:	

Certificate of Analysis

2010-L606-Rev.C

For Research Use Only

Product Description:

Taq DNA Ligase catalyzes the formation of a phosphodiester bond in duplex DNA containing adjacent 5'-phosphoryl and 3'-hydroxyl termini, using NAD⁺ as a cofactor.

Source of Protein

A recombinant *E. coli* strain carrying the cloned Taq DNA Ligase gene.

Supplied In

10 mM Tris-HCl
50 mM KCl
1 mM dithiothreitol
0.1 mM EDTA
0.1% Tween-20
50% glycerol
pH 7.5 @ 25°C

Supplied With

B606 10X Taq DNA Ligase Buffer

10X Taq DNA Ligase Buffer (B606)

200 mM Tris-HCl
250 mM Potassium Acetate
100 mM Magnesium Acetate
5 mM NAD⁺
0.1% Triton X-100
pH 7.6 @ 25°C

Unit Definition

1 unit is defined as the amount of Taq DNA Ligase required to join 50% of 1 µg of the 12-base cohesive ends of Lambda DNA cut with Hind III in 50 µl 1X Taq DNA Ligase Buffer following a 10 minute incubation at 45°C.

Product Specification*

Unit Size	20,000 Units
Unit Concentration	40,000 U/mL
Volume	0.5 mL
Purity (SDS-PAGE)	>99%
Specific Activity	400,000 U/mg
SS Exonuclease	400 U <5.0% released
DS Exonuclease	400 U <0.5% released
Endonuclease	400 U <10% converted
<i>E. coli</i> 16S rDNA Contamination	400 U <10 copies
Storage	-20°C

* For a detailed summary of assay conditions and data, refer to the Quality Controls Analysis section below

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 Taq DNA Ligase Reaction Buffer ([Taq DNA Ligase]_f = 0.1-0.0008 µg/µL) and added to 50 µL reactions containing 1.0 µg DNA and 1X Taq DNA Ligase Reaction Buffer. Reactions are incubated for 10 minutes at 45°C, stopped, and analyzed on a 0.8% agarose gel stained with ethidium bromide.

SDS-Page (Physical Purity Assessment)

2.0 µL of concentrated 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 same enzyme species. 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.

Nuclease Contamination Tests:

Single-Stranded Exonuclease Activity

A 50 µL reaction containing 11,000 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 5.0% release of TCA-soluble counts.

Double-Stranded Exonuclease Activity

A 50 µL reaction containing 5,000 cpm of a radiolabeled double-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C resulted in less than 0.5% release of TCA-soluble counts.

Endonuclease Activity

A 50 μ L reaction containing 0.5 μ g of pENZuC DNA and 10 μ L 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.

Real-Time PCR DNA Contamination Test:

Replicate 5 μ L 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.

Legal Disclaimers

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