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Certificate of Analysis

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E. coli DNA Ligase

Catalog No: 1065

Lot No: 2205010

Package Size: See Product Label

Concentration: 20 units/ μ l

Protein (Lowry): 0.088 mg/ml

Specific Activity: 228,571 units/mg

Storage Conditions: Store at -20°C

Unit Definition

One unit is the amount of enzyme required to yield 50% ligation of Hind III fragments of Lambda DNA at 16°C for 30 minutes in a 20 μ l assay mixture containing a DNA terminus concentration of 0.12 μM (300 $\mu\text{g}/\text{ml}$).

Assay Conditions

30 mM Tris-HCl (pH 8.0)

4 mM MgCl_2

1.2 mM EDTA

1 mM dithiothreitol (DTT)

0.026 mM NAD^+

Hind III fragments of Lambda DNA

Incubation at 16°C for 30 minutes

Storage Buffer

10 mM Tris-HCl (pH 7.4)

50 mM KCl

0.1 mM EDTA

10 mM ammonium sulfate

1 mM dithiothreitol

50% (v/v) glycerol

Quality Control

Nicking: Incubation of 5, 10, and 20 units of E coli DNA Ligase with 1.0 μg of pBR322 DNA for 1 hour at 37°C resulted in $\leq 5\%$ conversion of RFI to RFII DNA. Reaction volume is 50 μ l.

5'-Exonuclease/5'-Phosphatase:

Incubation of 5, 10, and 20 units of E. coli DNA Ligase with 0.25 μg of 5'-ends of [$5\text{-}^{32}\text{P}$] Lambda/Hae III DNA fragments for 1 hour at 37°C resulted in a ≤ 0.6 slope of %-end label released per unit of enzyme. Reaction volume is 50 μ l.

3'-Exonuclease: Incubation of 5, 10, and 20 units of E coli DNA Ligase with 5 pmoles of 3'-ends of Lambda/Taq I fragments (3'-labeled with Klenow exo- and [^3H]dCTP), incubated for 1 hour at 37°C resulted in a ≤ 0.6 slope of %-end label released per unit of enzyme. Reaction volume is 50 μ l.

DNase, double-stranded: Incubation of 5, 10, and 20 units of E. coli DNA Ligase with 0.015 μg of [^{32}P] Lambda DNA for 1 hour at 37°C resulted in ≤ 0.6 slope of %-end label released per unit of enzyme. Reaction volume is 20 μ l.

DNase, single-stranded: Incubation of 5, 10, and 20 units of E. coli DNA Ligase with 0.015 μg of heat denatured [^{32}P] Lambda DNA for 1 hour at 37°C resulted in ≤ 0.6 slope of %-end label released per unit of enzyme. Reaction volume of 20 μ l.

Purity: Approximately $\geq 95\%$ pure, as judged by SDS-polyacrylamide gel electrophoresis.