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Certificate of Analysis

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T4 DNA Ligase

Catalog No: 1060

Lot No: 2010013

Package Size: See Product Label

Concentration: 4 units/ μ l (Weiss units*)

*Note: 67 cohesive end ligation units are the equivalent of one Weiss unit.

Protein (Bradford): 0.53 mg/ml

Specific Activity: 7,489 units/mg

Incubation Temperature: 16°C - 22°C

Storage Conditions: Store at -20°C

Unit Definition

One unit is the amount of enzyme required to convert 1 nmole of ^{32}P from pyrophosphate into Norit-adsorbable form in 20 minutes at 37°C.

Reaction Conditions

66 mM Tris-HCl (pH 7.6)

6.7 mM MgCl_2

67 μM ATP

3.3 μM [^{32}P] $\text{Na}_4\text{P}_2\text{O}_7$ (1×10^8

CPM/ μmol)

10 mM dithiothreitol (DTT)

Storage Buffer

10 mM Tris-HCl (pH 7.5)

50 mM KCl

1 mM dithiothreitol (DTT)

50% (v/v) glycerol

Quality Control

Protein Concentration: Protein concentration was determined using the Bradford Assay.

Protein Purity: 50 ng, 100 ng, and 200 ng were evaluated by SDS-PAGE/silver staining.

Nicking: 5, 10, and 20 units of T4 DNA ligase were incubated with pBR322 DNA for 1 hour at 37°C.

DNase: 5, 10, and 20 units of T4 DNA Ligase were incubated with [^{32}P] labeled (λ) Lamda DNA (both single- and double-stranded) for 1 hour at 37°C.

5'-Exonuclease/5'-Phosphatase: 5, 10, and 20 units of T4 DNA ligase were incubated with [γ - ^{32}P] labeled (λ) Lambda/Hae III DNA fragments for 1 hour at 37°C.

3'-Exonuclease: 5, 10, and 20 units of T4 DNA Ligase were incubated with [^3H] labeled 3'-ends of Lambda/Taq I DNA fragments for 1 hour at 37°C.