T4 DNA Ligase

Catalog: E-102
Lot: 3

Quantity Supplied: 10 mg in 552 ul storage buffer.
Concentration: 18.1 ug/ul (highly concentrated)

Storage Buffer: 100 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1.0 mM EDTA, 200 mM imidazole, 10 mM dithiothreitol, 0.10% Triton X-100, 25% glycerol.

Purity: Greater than 98% as determined by SDS-PAGE gel analysis.

DNA Ligase Activity: approximately 600 cohesive end ligation units per ug protein using a qualitative blunt end dsDNA ligase assay (the enzyme activity per ug is roughly the same as the activity of New England Biolabs T4 DNA ligase, which was used for comparison).

Storage: -20 °C or below. Repeated freeze-thaw cycles have no effect on this product.

Description: T4 DNA ligase ligates adjacent 3'-hydroxyl and 5'-phosphate bases in double stranded DNA. T4 DNA ligase also ligates blunt ended dsDNA fragments. The recommended 10X buffer is 500 mM Tris-HCl, pH 7.5, 50 mM $MgCl_2$, 50 mM dithiothreitol, 10 mM ATP.

Recommended Use: This is a highly concentrated preparation of T4 DNA ligase. Typical ligation reactions can be easily accomplished by using a 100 fold (or more) dilution of this T4 DNA ligase preparation. The reaction should be extracted with an equal volume of phenol:$CHCl_3$, prior to agarose gel electrophoresis, in order to avoid electrophoretic artifacts.

Enzyme Stability: This enzyme preparation should be stored in a freezer, at -20 °C or lower in order to preserve enzyme activity long term. Stability experiments show that storage of this stock solution at 25 °C (room temperature) for 6 days results in a loss of about 25% of ligase activity. Storage at 37 °C for 6 days results in a loss of about 60% of ligase activity.

Quality Control Tests
(1) Endonuclease contamination: None detected.
Incubation of 54 ug (~ 32,000 units) with 15 ug of pUC19 supercoiled plasmid in 50 ul 50 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 5 mM dithiothreitol, at 37 °C for 3 hours resulted in no observable conversion of supercoiled plasmid to open circular form.
(2) Single Stranded Exonuclease: None detected.
Incubation of 54 ug (~ 32,000 units) with 110 ug of 43-mer oligonucleotide in 50 ul 50 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 5 mM dithiothreitol, at 37 °C for 3 hours resulted in no observable degradation of the 43-mer oligonucleotide.
(3) Double Stranded Exonuclease: None detected.
Incubation of 54 ug (~ 32,000 units) with 50 ug of a 20 base pair double stranded DNA ladder in 50 ul 50 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 5 mM dithiothreitol, at 37 °C for 3 hours resulted in no observable degradation of the 20 base pair dsDNA fragment, nor any of the upper DNA ladder bands.
(4) SDS-PAGE
SDS-PAGE analysis followed by coomassie blue staining shows only one major band. Purity is visually estimated at 98%. This product does not contain bovine serum albumin, or any other protein additives.

Made in USA by Edward Hyman

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