NEW ENGLAND BioLabs® Inc.

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New England Biolabs
Product Specification

Product Name:	*T3 DNA Ligase*
Catalog #:	*M0317S/L*
Concentration:	*3,000,000 units/ml*
Unit Definition:	*One unit is defined as the amount of enzyme required to give 50% ligation of 100 ng of Lambda-HindIII fragments in 1 minute at 25°C.*
Shelf Life:	*24 months*
Storage Temp:	*-20°C*
Storage Conditions:	*10 mM Tris-HCl , 50 mM KCl , 1 mM DTT , 0.1 mM EDTA , 50 % Glycerol, (pH 7.4 @ 25°C)*
Specification Version:	*PS-M0317S/L v1.0*
Effective Date:	*12 Dec 2016*

Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking) - A 50 µl reaction in NEBuffer 1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 15000 units of T3 DNA Ligase incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

Exonuclease Activity (Radioactivity Release) - A 50 µl reaction in NEBuffer 1 containing 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA and a minimum of 15000 units of T3 DNA Ligase incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

Functional Testing (Adaptor Ligation) - A 20 µl reaction in 1X T3 DNA Ligase Reaction Buffer containing 40 µM of phosphorylated linker and 3000 units of T3 DNA Ligase incubated for 16 hours at 16°C results in no detectable unligated adaptor as determined by agarose gel electrophoresis.

Ligation and Recutting (Terminal Integrity, Digested DNA) - A 20 µl reaction in 1X T3 DNA Ligase Reaction Buffer containing 2 µg of Lambda DNA-HindIII Digest and a minimum of 3000 units of T3 DNA Ligase incubated for 16 hours at 37°C results in >95% ligation of the DNA fragments as determined by agarose gel electrophoresis. Of these ligated fragments, >95% can be recut with HindIII.

Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in NEBuffer 1 containing 1 µg of CIP-treated Lambda-HindIII DNA and a minimum of 3000 units of T3 DNA Ligase incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Protein Concentration (A280) - The concentration of T3 DNA Ligase is 1 mg/ml +/- 10% as determined by UV absorption at 280 nm. Protein concentration is determined by the Pace method using the extinction coefficient of 62,130 and molecular weight of 39,351 daltons for T3 DNA Ligase (Pace, C.N. et al. (1995) Protein Sci., 4, 2411-2423).

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Protein Purity Assay (SDS-PAGE) - T3 DNA Ligase is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.
RNase Activity (Extended Digestion) - A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 µl of T3 DNA Ligase is incubated at 37°C. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.

Date 12 Dec 2016

Derek Robinson
Director of Quality Control