NEW ENGLAND BioLabs Inc.
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New England Biolabs
Certificate of Analysis

Product Name:	*T3 DNA Ligase*
Catalog Number:	*M0317L*
Concentration:	*3,000,000 U/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.*
Lot Number:	*10039217*
Expiration Date:	*03/2021*
Storage Temperature:	*-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*

T3 DNA Ligase Component List			
NEB Part Number	**Component Description**	**Lot Number**	**Individual QC Result**
M0317LVIAL	T3 DNA Ligase	10039218	**Pass**
B0317SVIAL	T3 DNA Ligase Buffer	10037888	**Pass**

Assay Name/Specification	**Lot # 10039217**
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 37ºC. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.	**Pass**
Protein Purity Assay (SDS-PAGE) T3 DNA Ligase is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	**Pass**
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).	**Pass**
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 37ºC results in a	**Pass**

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Assay Name/Specification	Lot # 10039217
DNA pattern free of detectable nuclease degradation 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.	**Pass**
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 16ºC results in no detectable unligated adaptor as determined by agarose gel electrophoresis.	**Pass**
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 37ºC results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	**Pass**
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 37ºC releases <0.1% of the total radioactivity.	**Pass**

This product has been tested and shown to be in compliance with all specifications.

Mary Lorenzen
Production Scientist
28 Feb 2019

Michael Tonello
Packaging Quality Control Inspector
19 Mar 2019