NEW ENGLAND BioLabs Inc.
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New England Biolabs
Certificate of Analysis

Product Name:	*T7 DNA Ligase*
Catalog Number:	*M0318L*
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 30 minutes at 25°C.*
Lot Number:	*10037316*
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-M0318S/L v1.0*

T7 DNA Ligase Component List			
NEB Part Number	**Component Description**	**Lot Number**	**Individual QC Result**
M0318LVIAL	T7 DNA Ligase	10037320	**Pass**
B0318SVIAL	T7 DNA Ligase Reaction Buffer	10020009	**Pass**

Assay Name/Specification	**Lot # 10037316**
Endonuclease Activity (Nicking) A 50 µl reaction in NEBuffer 1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 15000 units of T7 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 T7 DNA Ligase incubated for 4 hours at 37ºC releases <0.1% of the total radioactivity.	**Pass**
Ligation and Recutting (Terminal Integrity, Digested DNA) A 20 µl reaction in 1X T7 DNA Ligase Reaction Buffer containing 2 µg of Lambda DNA-HindIII Digest and a minimum of 3000 units of T7 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**
Non-Specific DNase Activity (16 Hour) A 50 µl reaction in NEBuffer 1 containing 1 µg of CIP-treated Lambda-HindIII DNA and	**Pass**

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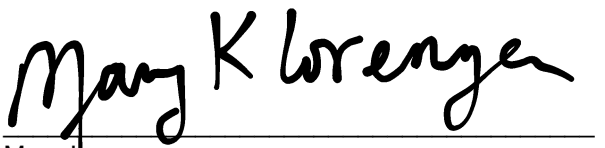
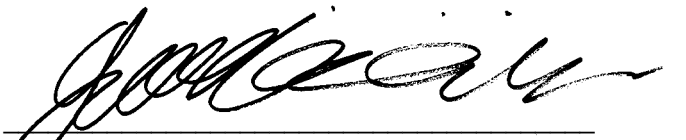
Assay Name/Specification	Lot # 10037316
a minimum of 3000 units of T7 DNA Ligase incubated for 16 hours at 37ºC results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	
Protein Concentration (A280) The concentration of T7 DNA Ligase is 1 mg/ml +/- 10% as determined by UV absorption at 280 nm. Protein concentration is determined by the Pace method using the extinction coefficient of 69,620 and molecular weight of 41,133 daltons for T7 DNA Ligase (Pace, C.N. et al. (1995) Protein Sci., 4, 2411-2423).	**Pass**
Protein Purity Assay (SDS-PAGE) T7 DNA Ligase is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	**Pass**
qPCR DNA Contamination (E. coli Genomic) A minimum of 3000 units of T7 DNA Ligase is screened for the presence of E. coli genomic DNA using SYBR® Green qPCR with primers specific for the E. coli 16S rRNA locus. Results are quantified using a standard curve generated from purified E. coli genomic DNA. The measured level of E. coli genomic DNA contamination is ≤ 1 E. coli genome.	**Pass**
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 T7 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**

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



Mary Lorenzen
Production Scientist
14 Sep 2018



Jay Minichiello
Packaging Quality Control Inspector
18 Mar 2019