NEW ENGLAND BioLabs Inc.

240 County Road
Ipswich, MA 01938-2723

Tel 978-927-5054
Fax 978-921-1350

www.neb.com
info@neb.com

New England Biolabs
Certificate of Analysis

Product Name:	*T7 DNA Ligase*
Catalog #:	*M0318S/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 30 minutes at 25°C.*
Lot #:	*0031803*
Assay Date:	*03/2018*
Expiration Date:	*3/2020*
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-M0318S/L v1.0*
Effective Date:	*03 Feb 2017*

Assay Name/Specification (minimum release criteria)	**Lot #0031803**
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 37°C 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 37°C 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 a minimum of 3000 units of T7 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.	**Pass**
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**

NEW ENGLAND BioLabs Inc.

240 County Road
Ipswich, MA 01938-2723

Tel 978-927-5054
Fax 978-921-1350

www.neb.com
info@neb.com

New England Biolabs
Certificate of Analysis

Assay Name/Specification (minimum release criteria)	**Lot #0031803**
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 37°C. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.	**Pass**

Authorized by
Derek Robinson
03 Feb 2017

Inspected by
Mary Lorenzen
15 Mar 2018

nqa ISO 9001 Registered Quality Management
nqa ISO 14001 Registered Environmental Management
nqa ISO 13485 Registered Medical Devices