NEW ENGLAND BioLabs Inc.
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New England Biolabs
Certificate of Analysis

Product Name:	*T4 DNA Ligase*
Catalog Number:	*M0202S*
Concentration:	*400,000 U/ml*
Unit Definition:	*One unit is defined as the amount of enzyme required to give 50% ligation of 6 µg of Lambda-HindIII DNA in 30 minutes at 16°C in a total reaction volume of 20 µl.*
Packaging Lot Number:	*10129940*
Expiration Date:	*07/2023*
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-M0202S/L v1.0*

T4 DNA Ligase Component List			
NEB Part Number	**Component Description**	**Lot Number**	**Individual QC Result**
M0202SVIAL	T4 DNA Ligase	10113858	**Pass**
B0202AVIAL	T4 DNA Ligase Reaction Buffer	10127256	**Pass**

Assay Name/Specification	**Lot # 10129940**
Ligation and Recutting (Terminal Integrity, Digested DNA) A 20 µl reaction in 1X T4 DNA Ligase Reaction Buffer containing 2 µg of Lambda DNA-HindIII Digest and a minimum of 4000 units of T4 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 2000 units of T4 DNA Ligase incubated for 16 hours at 37ºC results in a DNA pattern free of detectable nuclease degradation 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 2000 units of T4 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)	**Pass**

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Assay Name/Specification	Lot # 10129940
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 2000 units of T4 DNA Ligase incubated for 4 hours at 37ºC releases <0.1% of the total radioactivity.	
DNase Activity (Labeled Oligo, 5' extension) A 50 µl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent labeled double-stranded oligonucleotide containing a 5' extension and a minimum of 10,000 units of T4 DNA Ligase incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.	**Pass**
Double Stranded DNase Activity (Labeled Oligo) A 50 µl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent labeled double-stranded oligonucleotide containing a blunt end and a minimum of 10,000 units of T4 DNA Ligase incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.	**Pass**
DNase Activity (Labeled Oligo, 3' extension) A 50 µl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent labeled double-stranded oligonucleotide containing a 3' extension and a minimum of 10,000 units of T4 DNA Ligase incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.	**Pass**
Protein Concentration (A280) The concentration of T4 DNA Ligase is 0.4 mg/ml +/- 10% as determined by UV absorption at 280 nm. Protein concentration is determined by the Pace method using the extinction coefficient of 57,675 and molecular weight of 55,292 daltons for T4 DNA Ligase (Pace, C.N. et al. (1995) Protein Sci., 4, 2411-2423).	**Pass**
Protein Purity Assay (SDS-PAGE) T4 DNA Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	**Pass**
Single Stranded DNase Activity (FAM-Labeled Oligo) A 50 µl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent internal labeled oligonucleotide and a minimum of 10,000 units of T4 DNA Ligase incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.	**Pass**
qPCR DNA Contamination (E. coli Genomic) A minimum of 2000 units of T4 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	**Pass**

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genome. **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 T4 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.

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.

Ana Egana
Production Scientist
15 Nov 2021

Michael Tonello
Packaging Quality Control Inspector
15 Nov 2021