NEW ENGLAND BioLabs Inc.
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New England Biolabs
Certificate of Analysis

Product Name:	*Hi-T4™ DNA Ligase*
Catalog Number:	*M2622S*
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 25°C in a total reaction volume of 20 µl.*
Packaging Lot Number:	*10062171*
Expiration Date:	*11/2021*
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-M2622S/L v1.0*

Hi-T4™ DNA Ligase Component List			
NEB Part Number	**Component Description**	**Lot Number**	**Individual QC Result**
M2622SVIAL	Hi-T4™ DNA Ligase	10058609	**Pass**
B0535AVIAL	StickTogether™ DNA Ligase Buffer	10053903	**Pass**
B0202AVIAL	T4 DNA Ligase Reaction Buffer	10062811	**Pass**

Assay Name/Specification	**Lot # 10062171**
qPCR DNA Contamination (E. coli Genomic) A minimum of 400 units of Hi-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 genome.	**Pass**
Protein Purity Assay (SDS-PAGE) Hi-T4™ DNA Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	**Pass**
Protein Concentration (A280) The concentration of Hi-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 56,806 daltons for Hi-T4™ DNA Ligase (Pace, C.N. et al. (1995) Protein Sci., 4, 2411-2423).	**Pass**

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Assay Name/Specification	Lot # 10062171
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 2000 units of Hi-T4™ DNA Ligase incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.	**Pass**
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 2000 units of Hi-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 2000 units of Hi-T4™ DNA Ligase incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.	**Pass**
Endonuclease Activity (Nicking) A 50 µl reaction in NEBuffer 1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 400 units of Hi-T4™ DNA Ligase incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	**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 2000 units of Hi-T4™ DNA Ligase incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.	**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 400 units of Hi-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**
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 Hi-T4™ 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**

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

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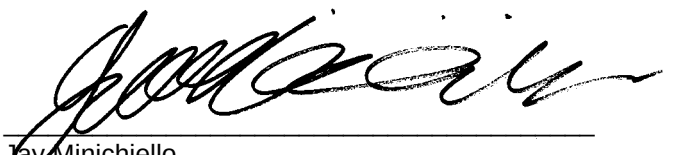
Mary Lorenzen
Production Scientist
07 Nov 2019



Jay Minichiello
Packaging Quality Control Inspector
21 Feb 2020