

## 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:** 10058734  
**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	10058376	Pass

Assay Name/Specification	Lot # 10058734
<p><b>Single Stranded DNase Activity (FAM-Labeled Oligo)</b>            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 &lt;5% degradation as determined by capillary electrophoresis.</p>	Pass
<p><b>RNase Activity (Extended Digestion)</b>            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, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</p>	Pass
<p><b>qPCR DNA Contamination (E. coli Genomic)</b>            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</p>	Pass

Assay Name/Specification	Lot # 10058734
genomic DNA. The measured level of E. coli genomic DNA contamination is $\leq 1$ E. coli genome.	
<p><b>Protein Purity Assay (SDS-PAGE)</b> Hi-T4™ DNA Ligase is <math>\geq 95\%</math> pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>
<p><b>Protein Concentration (A280)</b> 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).</p>	<b>Pass</b>
<p><b>Non-Specific DNase Activity (16 Hour)</b> A 50 <math>\mu</math>l reaction in NEBuffer 1 containing 1 <math>\mu</math>g of CIP-treated Lambda-HindIII DNA and a minimum of 400 units of Hi-T4™ 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.</p>	<b>Pass</b>
<p><b>Endonuclease Activity (Nicking)</b> A 50 <math>\mu</math>l reaction in NEBuffer 1 containing 1 <math>\mu</math>g of supercoiled PhiX174 DNA and a minimum of 400 units of Hi-T4™ DNA Ligase incubated for 4 hours at 37°C results in &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	<b>Pass</b>
<p><b>Double Stranded DNase Activity (Labeled Oligo)</b> A 50 <math>\mu</math>l 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 &lt;5% degradation as determined by capillary electrophoresis.</p>	<b>Pass</b>
<p><b>DNase Activity (Labeled Oligo, 5' extension)</b> A 50 <math>\mu</math>l 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 &lt;5% degradation as determined by capillary electrophoresis.</p>	<b>Pass</b>
<p><b>DNase Activity (Labeled Oligo, 3' extension)</b> A 50 <math>\mu</math>l 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 &lt;5% degradation as determined by capillary electrophoresis.</p>	<b>Pass</b>

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

*Mary K Lorenzen*

Mary Lorenzen  
Production Scientist  
09 Sep 2019

*Michael Tonello*

Michael Tonello  
Packaging Quality Control Inspector  
11 Nov 2019