

## New England Biolabs Certificate of Analysis

*Product Name:* T4 DNA Ligase  
*Catalog #:* M0202T/M  
*Concentration:* 2,000,000 units/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.  
*Lot #:* 1281804  
*Assay Date:* 04/2018  
*Expiration Date:* 4/2020  
*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-M0202T/M v1.0  
*Effective Date:* 01 Dec 2017

Assay Name/Specification (minimum release criteria)	Lot #1281804
<b>DNase Activity (Labeled Oligo, 3' extension)</b> - 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.	<b>Pass</b>
<b>DNase Activity (Labeled Oligo, 5' extension)</b> - 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.	<b>Pass</b>
<b>Double Stranded DNase Activity (Labeled Oligo)</b> - 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.	<b>Pass</b>
<b>Endonuclease Activity (Nicking)</b> - 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 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	<b>Pass</b>
<b>Exonuclease Activity (Radioactivity Release)</b> - A 50 µl reaction in NEBuffer 1 containing 1 µg of a mixture of single and double-stranded [ <sup>3</sup> H] <i>E. coli</i> DNA and a minimum of 2000 units of T4 DNA Ligase incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	<b>Pass</b>

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Assay Name/Specification (minimum release criteria)	Lot #1281804
<p><b>Ligation and Recutting (Terminal Integrity, Digested DNA)</b> - 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 &gt;95% ligation of the DNA fragments as determined by agarose gel electrophoresis. Of these ligated fragments, &gt;95% can be recut with HindIII.</p>	<b>Pass</b>
<p><b>Non-Specific DNase Activity (16 Hour)</b> - 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 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.</p>	<b>Pass</b>
<p><b>Protein Concentration (A280)</b> - The concentration of T4 DNA Ligase is 2 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).</p>	<b>Pass</b>
<p><b>Protein Purity Assay (SDS-PAGE)</b> - T4 DNA Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>
<p><b>qPCR DNA Contamination (E. coli Genomic)</b> - A minimum of 2000 units of T4 DNA Ligase is screened for the presence of <i>E. coli</i> genomic DNA using SYBR® Green qPCR with primers specific for the <i>E. coli</i> 16S rRNA locus. Results are quantified using a standard curve generated from purified <i>E. coli</i> genomic DNA. The measured level of <i>E. coli</i> genomic DNA contamination is ≤ 1 <i>E. coli</i> genome.</p>	<b>Pass</b>
<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 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>	<b>Pass</b>
<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 10,000 units of T4 DNA Ligase incubated for 16 hours at 37°C yields &lt;5% degradation as determined by capillary electrophoresis.</p>	<b>Pass</b>



Authorized by  
Derek Robinson  
01 Dec 2017



Inspected by  
Mary Lorenzen  
06 Apr 2018

