

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: 10077023
Expiration Date: 01/2022
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

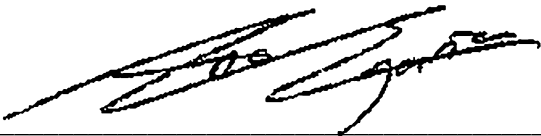
T4 DNA Ligase Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0202SVIAL	T4 DNA Ligase	10064879	Pass
B0202AVIAL	T4 DNA Ligase Reaction Buffer	10069975	Pass

Assay Name/Specification	Lot # 10077023
<p>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.</p>	Pass
<p>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 37°C. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</p>	Pass
<p>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.</p>	Pass

Assay Name/Specification	Lot # 10077023
<p>Exonuclease Activity (Radioactivity Release) A 50 µl reaction in NEBuffer 1 containing 1 µg of a mixture of single and double-stranded [³H] E. coli 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.</p>	Pass
<p>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 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	Pass
<p>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.</p>	Pass
<p>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.</p>	Pass
<p>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.</p>	Pass
<p>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 genome.</p>	Pass
<p>Protein Purity Assay (SDS-PAGE) T4 DNA Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	Pass
<p>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</p>	Pass

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<p>DNA Ligase (Pace, C.N. et al. (1995) Protein Sci., 4, 2411-2423).</p> <p>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 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.</p>	<p>Pass</p>

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



Ana Egana
Production Scientist
13 Jul 2020



Michael Tonello
Packaging Quality Control Inspector
13 Jul 2020