

## New England Biolabs Certificate of Analysis

**Product Name:** *E.coli DNA Ligase*  
**Catalog Number:** M0205S  
**Concentration:** 10,000 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:** 10215588  
**Expiration Date:** 11/2025  
**Storage Temperature:** -20°C  
**Storage Conditions:** 10 mM Tris-HCl , 50 mM KCl , 1 mM DTT , 0.1 mM EDTA , 200 µg/ml BSA , 50 % Glycerol, (pH 7.4 @ 25°C)  
**Specification Version:** PS-M0205S/L v2.0

E.coli DNA Ligase Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0205SVIAL	E.coli DNA Ligase	10211795	Pass
B0205SVIAL	E. coli DNA Ligase Reaction Buffer	10168548	Pass

Assay Name/Specification	Lot # 10215588
<b>Endonuclease Activity (Nicking)</b> A 50 µl reaction in CutSmart® Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 50 units of E. coli DNA Ligase incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
<b>Exonuclease Activity (Radioactivity Release)</b> A 50 µl reaction in CutSmart® Buffer containing 1 µg of a mixture of single and double-stranded [ <sup>3</sup> H] E. coli DNA and a minimum of 50 units of E. coli DNA Ligase incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
<b>Non-Specific DNase Activity (16 Hour)</b> A 50 µl reaction in CutSmart® Buffer containing 1 µg of CIP-treated Lambda-HindIII DNA and a minimum of 20 units of E. coli 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.	Pass
<b>Protein Purity Assay (SDS-PAGE)</b> E. coli DNA Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie	Pass

Assay Name/Specification	Lot # 10215588
<p>Blue detection.</p> <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 E. coli 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> <p><b>qPCR DNA Contamination (E. coli Genomic)</b> A minimum of 10 units of E. coli 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>	<p><b>Pass</b></p> <p><b>Pass</b></p>

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

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