

## New England Biolabs Certificate of Analysis

**Product Name:** *E.coli DNA Ligase*  
**Catalog Number:** M0205L  
**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:** 10132241  
**Expiration Date:** 09/2023  
**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 |
| M0205LVIAL                       | E.coli DNA Ligase                  | 10118369   | Pass                 |
| B0205SVIAL                       | E. coli DNA Ligase Reaction Buffer | 10102315   | Pass                 |

| Assay Name/Specification   | Lot # 10132241 |
|--|----------------|
| <p><b>qPCR DNA Contamination (E. coli Genomic)</b><br/>           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> | Pass           |
| <p><b>RNase Activity (Extended Digestion)</b><br/>           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>  | Pass           |
| <p><b>Protein Purity Assay (SDS-PAGE)</b><br/>           E. coli DNA Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>   | Pass           |
| <p><b>Endonuclease Activity (Nicking)</b></p>  | Pass           |

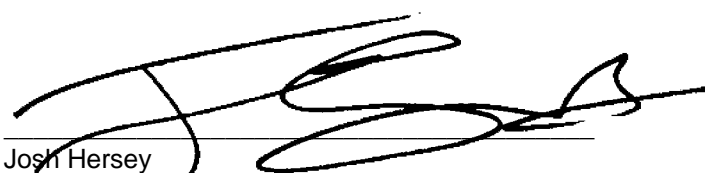
| Assay Name/Specification   | Lot # 10132241 |
|--|----------------|
| <p>A 50 µl reaction in CutSmart® Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 50 units of E. coli DNA Ligase incubated for 4 hours at 37°C results in &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p>  |                |
| <p><b>Exonuclease Activity (Radioactivity Release)</b><br/>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 &lt;0.1% of the total radioactivity.</p>                       | <b>Pass</b>    |
| <p><b>Non-Specific DNase Activity (16 Hour)</b><br/>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.</p> | <b>Pass</b>    |

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

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07 Dec 2021



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