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## 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: 10094095
Expiration Date: 12/2022
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 |                                    |            |                      |  |
|----------------------------------|------------------------------------|------------|----------------------|--|
| <b>NEB Part Number</b>           | Component Description              | Lot Number | Individual QC Result |  |
| M0205LVIAL                       | E.coli DNA Ligase                  | 10094100   | Pass                 |  |
| B0205SVIAL                       | E. coli DNA Ligase Reaction Buffer | 10067298   | Pass                 |  |

| Assay Name/Specification  | Lot # 10094095 |
|---|----------------|
| Endonuclease Activity (Nicking) 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           |
| Exonuclease Activity (Radioactivity Release) A 50 μl reaction in CutSmart® Buffer containing 1 μg of a mixture of single and double-stranded [ ³H] 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           |
| 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 E. coli 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. | Pass           |
| qPCR DNA Contamination (E. coli Genomic) A minimum of 10 units of E. coli DNA Ligase is screened for the presence of E. coli  | Pass           |



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| Assay Name/Specification   | Lot # 10094095 |  |
|--|----------------|--|
| 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.   |                |  |
| Protein Purity Assay (SDS-PAGE) E. coli DNA Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.  | Pass           |  |
| Non-Specific DNase Activity (16 Hour) 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           |  |

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

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.

Ana Egana Production Scientist

06 Jan 2021

Josh Hersey

Packaging Quality Control Inspector

06 Jan 2021



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