

be INSPIRED *drive* DISCOVERY *stay* GENUINE

240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

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 μ g of Lambda-HindIII DNA in 30 minutes at 16°C in a total reaction volume of 20 μ l. |
| Lot Number: | 10028144 |
| Expiration Date: | 11/2020 |
| 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 | 10028141 | Pass | |
| B0205SVIAL | E. coli DNA Ligase Reaction Buffer | 0021711 | Pass | |

| Assay Name/Specification | Lot # 10028144 |
|---|----------------|
| 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 [³ 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 |
| 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 |
| Protein Purity Assay (SDS-PAGE) E. coli DNA Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie | Pass |





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| Assay Name/Specification | Lot # 10028144 |
|---|----------------|
| Blue detection. | |
| qPCR DNA Contamination (E. coli Genomic) 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. | 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 |

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

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Mary Lorenzen Production Scientist 15 Nov 2018

Michae 11.

Michael Tonello Packaging Quality Control Inspector 15 Nov 2018

