240 County Road
Ipswich, MA 01938-2723
Tel 978-927-5054
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www.neb.com
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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:	*10246032*
Expiration Date:	*03/2026*
Storage Temperature:	*-20°C*
Storage Conditions:	*10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 200 µg/ml rAlbumin, 50 % Glycerol, (pH 7.4 @ 25°C)*
Specification Version:	*PS-M0205S/L v3.0*

E.coli DNA Ligase Component List			
NEB Part Number	**Component Description**	**Lot Number**	**Individual QC Result**
M0205LVIAL	E.coli DNA Ligase	10232140	**Pass**
B0205SVIAL	E. coli DNA Ligase Reaction Buffer	10233153	**Pass**

Assay Name/Specification	**Lot # 10246032**
Endonuclease Activity (Nicking) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of supercoiled PhiX174 RF I 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 rCutSmart™ 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 rCutSmart™ 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 37ºC results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	**Pass**
Protein Purity Assay (SDS-PAGE)	**Pass**

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Assay Name/Specification	Lot # 10246032
E. coli DNA Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	
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 37ºC. 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 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**

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

Alison Dolan
Production Scientist
07 Mar 2024

Josh Hersey
Packaging Quality Control Inspector
05 Jul 2024