NEW ENGLAND BioLabs Inc.
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240 County Road
Ipswich, MA 01938-2723
Tel 978-927-5054
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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.*
Lot Number:	*10053290*
Expiration Date:	*06/2021*
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	10045746	**Pass**
B0205SVIAL	E. coli DNA Ligase Reaction Buffer	10029823	**Pass**

Assay Name/Specification	**Lot # 10053290**
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**
Protein Purity Assay (SDS-PAGE) E. coli DNA Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	**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 37ºC. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.	**Pass**
Non-Specific DNase Activity (16 Hour)	**Pass**

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Assay Name/Specification	Lot # 10053290
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 37ºC results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	
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 37ºC releases <0.1% of the total radioactivity.	**Pass**
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 37ºC results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	**Pass**

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

Mary Lorenzen
Production Scientist
25 Jan 2019

Michael Tonello
Packaging Quality Control Inspector
15 Aug 2019