

## New England Biolabs Certificate of Analysis

**Product Name:** *E. coli* DNA Ligase  
**Catalog #:** M0205S/L  
**Concentration:** 10,000 units/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 #:** 0911804  
**Assay Date:** 04/2018  
**Expiration Date:** 4/2020  
**Storage Temp:** -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  
**Effective Date:** 03 Feb 2017

Assay Name/Specification (minimum release criteria)	Lot #0911804
<b>Endonuclease Activity (Nicking)</b> - A 50 µl reaction in CutSmart® Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 50 units of <i>E. coli</i> DNA Ligase incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	<b>Pass</b>
<b>Exonuclease Activity (Radioactivity Release)</b> - A 50 µl reaction in CutSmart® Buffer containing 1 µg of a mixture of single and double-stranded [ <sup>3</sup> H] <i>E. coli</i> DNA and a minimum of 50 units of <i>E. coli</i> DNA Ligase incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	<b>Pass</b>
<b>Non-Specific DNase Activity (16 Hour)</b> - A 50 µl reaction in CutSmart® Buffer containing 1 µg of CIP-treated Lambda-HindIII DNA and a minimum of 20 units of <i>E. coli</i> 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.	<b>Pass</b>
<b>Protein Purity Assay (SDS-PAGE)</b> - <i>E. coli</i> DNA Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	<b>Pass</b>
<b>qPCR DNA Contamination (<i>E. coli</i> Genomic)</b> - A minimum of 10 units of <i>E. coli</i> DNA Ligase is screened for the presence of <i>E. coli</i> genomic DNA using SYBR® Green qPCR with primers specific for the <i>E. coli</i> 16S rRNA locus. Results are quantified using a standard curve generated from purified <i>E. coli</i> genomic DNA. The measured level of <i>E. coli</i> genomic DNA contamination is ≤ 1 <i>E. coli</i> genome.	<b>Pass</b>
<b>RNase Activity (Extended Digestion)</b> - A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 µl of <i>E. coli</i> 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.	<b>Pass</b>



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\* The BSA in this product has been granted an EDQM "Certificate of Suitability" from the European Directorate for the Quality of Medicines (# R1-CEP-2003-204-Rev00) and has been granted a USDA Certificate for Export of Bovine Blood Plasma/Serum for Manufacture into Pharmaceutical Products.



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Authorized by  
Derek Robinson  
03 Feb 2017



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Inspected by  
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03 Apr 2018

