

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 #: 0911703
Assay Date: 03/2017
Expiration Date: 3/2019
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 #0911703
Endonuclease Activity (Nicking) - 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.	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] <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.	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 <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.	Pass
Protein Purity Assay (SDS-PAGE) - <i>E. coli</i> DNA Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
qPCR DNA Contamination (<i>E. coli</i> Genomic) - 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.	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 <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.	Pass



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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.



Authorized by
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03 Feb 2017



Inspected by
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18 Apr 2017

