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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:	Quick Ligation™ Kit
Catalog Number:	M2200S
Unit Definition:	N/A
Packaging Lot Number:	10056656
Expiration Date:	08/2021
Storage Temperature:	-20°C
Storage Conditions:	10 mM Tris-HCl , 50 mM KCl , 1 mM DTT , 0.1 mM EDTA , 50 % Glycerol, (pH 7.4 @ 25°C)
Specification Version:	PS-M2200S/L v1.0

Quick Ligation™ Kit Component List				
NEB Part Number	Component Description	Lot Number	Individual QC Result	
M2200SVIAL	Quick Ligation™ Kit	10050901	Pass	
B2200SVIAL	Quick Ligation™ Reaction Buffer	10031305	Pass	

Assay Name/Specification	Lot # 10056656
<b>Single Stranded DNase Activity (FAM-Labeled Oligo)</b> A 50 µl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent internal labeled oligonucleotide and a minimum of 10,000 units of Quick Ligase incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.	Pass
<b>RNase Activity (Extended Digestion)</b> A 10 $\mu$ I reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 $\mu$ I of Quick 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
<b>qPCR DNA Contamination (E. coli Genomic)</b> A minimum of 2000 units of Quick 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) Quick Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue	Pass





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Assay Name/Specification	Lot # 10056656
detection.	
DNase Activity (Labeled Oligo, 3' extension) A 50 µl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent labeled double-stranded oligonucleotide containing a 3' extension and a minimum of 10,000 units of Quick Ligase incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.	Pass
DNase Activity (Labeled Oligo, 5' extension) A 50 µl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent labeled double-stranded oligonucleotide containing a 5' extension and a minimum of 10,000 units of Quick Ligase incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.	Pass
<b>Double Stranded DNase Activity (Labeled Oligo)</b> A 50 µl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent labeled double-stranded oligonucleotide containing a blunt end and a minimum of 10,000 units of Quick Ligase incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.	Pass
<b>Endonuclease Activity (Nicking)</b> A 50 µl reaction in NEBuffer 1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 2000 units of Quick Ligase incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
<b>Exonuclease Activity (Radioactivity Release)</b> A 50 μl reaction in NEBuffer 1 containing 1 μg of a mixture of single and double-stranded [ <sup>3</sup> H] E. coli DNA and a minimum of 2000 units of Quick Ligase incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
<b>Functional Testing (Ligation and Transformation)</b> After a five-minute ligation of linearized, dephosphorylated LITMUS 28 or pUC19 (containing either blunt [EcoRV] or cohesive [HindIII] ends) and a mixture of compatible insert fragments, transformation into chemically competent E. coli DH-5 alpha cells yields a minimum of 1 x 10e6 recombinant transformants per µg plasmid DNA.	Pass
<b>Ligation and Recutting (Terminal Integrity, Digested DNA)</b> A 20 µl reaction in 1X T4 DNA Ligase Reaction Buffer containing 2 µg of Lambda DNA-HindIII Digest and a minimum of 4000 units of Quick Ligase incubated for 16 hours at 37°C results in >95% ligation of the DNA fragments as determined by agarose gel electrophoresis. Of these ligated fragments, >95% can be recut with HindIII.	Pass





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Assay Name/Specification	Lot # 10056656
Non-Specific DNase Activity (16 Hour)	Pass
A 50 µl reaction in NEBuffer 1 containing 1 µg of CIP-treated Lambda-HindIII DNA and	
a minimum of 2000 units of Quick Ligase incubated for 16 hours at 37°C results in a	
DNA pattern free of detectable nuclease degradation as determined by agarose gel	
electrophoresis.	

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

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Mary Lorenzen Production Scientist 28 Mar 2019

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Minichiello Packaging Quality Control Inspector 13 Nov 2019

