

## New England Biolabs Certificate of Analysis

**Product Name:** Quick Ligation™ Kit  
**Catalog Number:** M2200L  
**Unit Definition:** N/A  
**Packaging Lot Number:** 10166734  
**Expiration Date:** 09/2024  
**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
M2200LVIAL	Quick Ligation™ Kit	10160077	Pass
B2200SVIAL	Quick Ligation™ Reaction Buffer	10166737	Pass

Assay Name/Specification	Lot # 10166734
<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>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
<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 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>Protein Purity Assay (SDS-PAGE)</b> Quick Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue	Pass

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detection.	
<p><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 &gt;95% ligation of the DNA fragments as determined by agarose gel electrophoresis. Of these ligated fragments, &gt;95% can be recut with HindIII.</p>	<b>Pass</b>
<p><b>Non-Specific DNase Activity (16 Hour)</b> 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.</p>	<b>Pass</b>
<p><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 10<sup>6</sup> recombinant transformants per µg plasmid DNA.</p>	<b>Pass</b>
<p><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 &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	<b>Pass</b>
<p><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 &lt;0.1% of the total radioactivity.</p>	<b>Pass</b>
<p><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 &lt;5% degradation as determined by capillary electrophoresis.</p>	<b>Pass</b>
<p><b>DNase Activity (Labeled Oligo, 5' extension)</b> 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 &lt;5% degradation as determined by capillary electrophoresis.</p>	<b>Pass</b>

Assay Name/Specification	Lot # 10166734
<p><b>DNase Activity (Labeled Oligo, 3' extension)</b> 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 &lt;5% degradation as determined by capillary electrophoresis.</p>	<p><b>Pass</b></p>

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

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