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New England Biolabs Certificate of Analysis

Product Name: Quick Ligation™ Kit

Catalog Number: M2200S
Unit Definition: N/A

Packaging Lot Number: 10080116
Expiration Date: 03/2022
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 | 10069172 | Pass | |
| B2200SVIAL | Quick Ligation™ Reaction Buffer | 10076213 | Pass | |

| Assay Name/Specification | Lot # 10080116 |
|--|----------------|
| 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 |
| Double Stranded DNase Activity (Labeled Oligo) 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 |
| Endonuclease Activity (Nicking) 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% | Pass |



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BioLabs inc.

| Assay Name/Specification | Lot # 10080116 |
|---|----------------|
| conversion to the nicked form as determined by agarose gel electrophoresis. | |
| Exonuclease Activity (Radioactivity Release) A 50 µl reaction in NEBuffer 1 containing 1 µg of a mixture of single and double-stranded [³H] 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 |
| Functional Testing (Ligation and Transformation) 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 |
| Ligation and Recutting (Terminal Integrity, Digested DNA) 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 |
| Non-Specific DNase Activity (16 Hour) 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. | Pass |
| Protein Purity Assay (SDS-PAGE) Quick Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection. | Pass |
| qPCR DNA Contamination (E. coli Genomic) 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 |
| 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 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 |



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| Assay Name/Specification | Lot # 10080116 |
|---|----------------|
| Single Stranded DNase Activity (FAM-Labeled Oligo) 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 |

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

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Ana Egana Production Scientist 09 Sep 2020 Michael Tonello

Packaging Quality Control Inspector 09 Sep 2020

