NEW ENGLAND BioLabs Inc.

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# New England Biolabs
# Certificate of Analysis

| | |
|---|---|
| *Product Name:* | *Quick Ligation™ Kit* |
| *Catalog #:* | *M2200S/L* |
| *Concentration:* | *1 reaction/µl* |
| *Unit Definition:* | *N/A* |
| *Lot #:* | *1281801* |
| *Assay Date:* | *01/2018* |
| *Expiration Date:* | *01/2020* |
| *Storage Temp:* | *-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* |
| *Effective Date:* | *07 Feb 2018* |

| **Assay Name/Specification** (minimum release criteria) | **Lot #1281801** |
|---|---|
| **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% conversion to the nicked form as determined by agarose gel electrophoresis. | **Pass** |
| **Exonuclease Activity (Radioactivity Release) -** A 50 µl reaction in NEBuffer 1 containing 1 µg of a mixture of single and double-stranded [ $^{3}$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** |

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| **Assay Name/Specification** (minimum release criteria) | **Lot #1281801** |
|---|---|
| **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** |
| **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** |

Authorized by
Derek Robinson
07 Feb 2018

Inspected by
Mary Lorenzen
14 Feb 2018

nqa ISO 9001 Registered Quality Management
nqa ISO 14001 Registered Environmental Management
nqa ISO 13485 Registered Medical Devices