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## New England Biolabs Product Specification

Product Name: Quick Ligation<sup>TM</sup> Kit

Catalog #: M2200S/L
Concentration: 1 reaction/µl

Unit Definition:N/AShelf Life:24 monthsStorage Temp: $-20^{\circ}C$ 

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)

DNase Activity (Labeled Oligo, 3' extension) - A 50  $\mu$ l 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.

DNase Activity (Labeled Oligo, 5' extension) - A 50  $\mu$ l 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.

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.

Endonuclease Activity (Nicking) - A 50  $\mu$ l reaction in NEBuffer 1 containing 1  $\mu$ g 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.

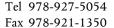
Exonuclease Activity (Radioactivity Release) - A 50  $\mu$ l reaction in NEBuffer 1 containing 1  $\mu$ g of a mixture of single and double-stranded [  $^3$ H] *E. coli* DNA and a minimum of 2000 units of Quick Ligase incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

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.









Date

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Ligation and Recutting (Terminal Integrity, Digested DNA) - A 20  $\mu$ l reaction in 1X T4 DNA Ligase Reaction Buffer containing 2  $\mu$ g 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.

Non-Specific DNase Activity (16 Hour) - A 50  $\mu$ l reaction in NEBuffer 1 containing 1  $\mu$ g 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.

Protein Purity Assay (SDS-PAGE) - Quick Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

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  $\leq 1$  *E. coli* genome.

RNase Activity (Extended Digestion) - A 10  $\mu$ l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1  $\mu$ l 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.

Single Stranded DNase Activity (FAM-Labeled Oligo) - A 50  $\mu$ l 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.

Derek Robinson

Director of Quality Control





