NEW ENGLAND BioLabs Inc.

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# New England Biolabs
# Certificate of Analysis

| | |
|---|---|
| *Product Name:* | *HiFi Taq DNA Ligase* |
| *Catalog #:* | *M0647S* |
| *Concentration:* | *1 reaction/µl* |
| *Unit Definition:* | *N/A* |
| *Lot #:* | *0021707* |
| *Assay Date:* | *07/2017* |
| *Expiration Date:* | *07/2019* |
| *Storage Temp:* | *-20°C* |
| *Storage Conditions:* | *10 mM Tris-HCl , 100 mM KCl , 1 mM DTT , 0.1 mM EDTA , 0.1 % Triton®X-100 , 50 % Glycerol, (pH 7.4 @ 25° C)* |
| *Specification Version:* | *PS-M0647S v3.0* |
| *Effective Date:* | *16 Apr 2018* |

| **Assay Name/Specification** (minimum release criteria) | **Lot #0021707** |
|---|---|
| **Endonuclease Activity (Nicking) -** A 50 µl reaction in NEBuffer 4 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 10 µl of HiFi *Taq* DNA 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 HiFi *Taq* DNA Ligase Buffer containing 1 µg of a mixture of single and double-stranded [ $^{3}$H] *E. coli* DNA and a minimum of 10 µl of HiFi *Taq* DNA Ligase incubated for 4 hours at 37°C releases <0.1% of the total radioactivity. | **Pass** |
| **Functional Testing (HiFi *Taq* DNA Ligase, Activity) -** A 20 µl reaction in HiFi *Taq* DNA Ligase Buffer containing 0.1 µM of a FAM-labeled nicked dsDNA substrate and 44 pM HiFi *Taq* DNA Ligase incubated for 10 minutes at 65°C results in 40%+/-20 ligation of the substrate as determined by capillary electrophoresis. | **Pass** |
| **Functional Testing (HiFi *Taq* DNA Ligase, Fidelity) -** A 20 µl reaction in HiFi *Taq* DNA Ligase Buffer containing 0.1 µM of an equimolar mix of a FAM-labeled nicked dsDNA substrate and 44 pM HiFi *Taq* DNA Ligase incubated for 10 minutes at 65°C results in complete ligation of the matched product and <40% ligation of the mismatched products as determined by capillary electrophoresis. | **Pass** |
| **Non-Specific DNase Activity (16 Hour) -** A 50 µl reaction in NEBuffer 4 containing 1 µg of Lambda-HindIII DNA and a minimum of 2 µl of HiFi *Taq* DNA 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** |

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| **Assay Name/Specification** (minimum release criteria) | **Lot #0021707** |
|---|---|
| **qPCR DNA Contamination (*E. coli* Genomic)** - A minimum of 1 µl of HiFi *Taq* DNA 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 HiFi *Taq* DNA Ligase is incubated at 37°C. After incubation for 4 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection. | **Pass** |

Authorized by
Derek Robinson
16 Apr 2018

Inspected by
Mary Lorenzen
25 Jul 2017

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