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New England Biolabs Product Specification

Product Name: HiFi Taq DNA Ligase

Catalog #: M0647S
Concentration: 1 reaction/µl

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

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 v4.0

Effective Date: 28 Oct 2024

Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking) - A 50 μ l reaction in NEBuffer 4 containing 1 μ g of supercoiled PhiX174 DNA and a minimum of 10 μ l 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.

Exonuclease Activity (Radioactivity Release) - A 50 μ l reaction in HiFi Taq DNA Ligase Buffer containing 1 μ g of a mixture of single and double-stranded [3 H] E. coli DNA and a minimum of 10 μ l of HiFi Taq DNA Ligase incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

Functional Testing (HiFi Taq DNA Ligase, Fidelity) - A 50 μ l reaction in HiFi Taq DNA Ligase Buffer containing 0.1 μ M of an equimolar mix of a FAM-labeled nicked dsDNA substrate and 1 μ l 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.

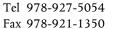
Non-Specific DNase Activity (16 Hour) - A 50 μ l reaction in NEBuffer 4 containing 1 μ g of Lambda-HindIII DNA and a minimum of 2 μ l of HiFi Taq DNA Ligase incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 1 μ l 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.









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Assay Name/Specification (minimum release criteria)

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

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 0.02 µl HiFi *Taq* DNA Ligase incubated for 10 minutes at 65°C results in 40%+/-20 ligation of the substrate as determined by capillary electrophoresis.

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Date 28 Oct 2024

Lauren Brown Quality Approver





