

New England Biolabs Product Specification

<i>Product Name:</i>	<i>HiFi Taq DNA Ligase</i>
<i>Catalog #:</i>	<i>M0647S</i>
<i>Concentration:</i>	<i>1 reaction/μl</i>
<i>Unit Definition:</i>	<i>N/A</i>
<i>Shelf Life:</i>	<i>24 months</i>
<i>Storage Temp:</i>	<i>-20°C</i>
<i>Storage Conditions:</i>	<i>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)</i>
<i>Specification Version:</i>	<i>PS-M0647S v4.0</i>
<i>Effective Date:</i>	<i>28 Oct 2024</i>

Assay Name/Specification (minimum release criteria)

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.

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 [³H] *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.

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

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.

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Assay Name/Specification (minimum release criteria)

<p>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 <i>Taq</i> 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.</p>

<p>Functional Testing (HiFi <i>Taq</i> DNA Ligase, Activity) - A 20 µl reaction in HiFi <i>Taq</i> DNA Ligase Buffer containing 0.1 µM of a FAM-labeled nicked dsDNA substrate and 0.02 µl HiFi <i>Taq</i> DNA Ligase incubated for 10 minutes at 65°C results in 40%+/-20 ligation of the substrate as determined by capillary electrophoresis.</p>

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Date 28 Oct 2024

Lauren Brown
Quality Approver

