

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 v3.0</i>
<i>Effective Date:</i>	<i>16 Jan 2018</i>

Assay Name/Specification (minimum release criteria)
<p>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.</p> <p>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] <i>E. coli</i> 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.</p> <p>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.</p> <p>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.</p> <p>qPCR DNA Contamination (<i>E. coli</i> Genomic) - A minimum of 1 μl of HiFi Taq DNA Ligase is screened for the presence of <i>E. coli</i> genomic DNA using SYBR® Green qPCR with primers specific for the <i>E. coli</i> 16S rRNA locus. Results are quantified using a standard curve generated from purified <i>E. coli</i> genomic DNA. The measured level of <i>E. coli</i> genomic DNA contamination is ≤ 1 <i>E. coli</i> genome.</p> <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 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.</p>



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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.



Date 16 Jan 2018

Derek Robinson
Director of Quality Control

