New England Biolabs
Product Specification

**Product Name:** EcoRI-HF®

**Catalog #:** R3101T/M

**Concentration:** 100,000 units/ml

**Unit Definition:** One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA in rCutSmart Buffer in 1 hour at 37°C in a total reaction volume of 50 µl.

**Shelf Life:** 24 months

**Storage Temp:** -20°C

**Storage Conditions:** 300 mM NaCl, 10 mM KPO₄, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, 0.15 % Triton X-100, 200 µg/ml rAlbumin, (pH 7.0 @ 25°C)

**Specification Version:** PS-R3101T/M v3.0

**Effective Date:** 07 Nov 2023

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### Assay Name/Specification (minimum release criteria)

**Blue-White Screening (Terminal Integrity)** - A sample of pUC19 vector linearized with a 10-fold excess of EcoRI-HF®, religated and transformed into an *E. coli* strain expressing the LacZ beta fragment gene results in <1% white colonies.

**Endonuclease Activity (Nicking)** - A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 100 units of EcoRI-HF® 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 rCutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [³H] *E. coli* DNA and a minimum of 200 units of EcoRI-HF® incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

**Ligation and Recutting (Terminal Integrity)** - After a 20-fold over-digestion of Lambda DNA with EcoRI-HF®, >95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, >95% can be recut with EcoRI-HF®.

**Non-Specific DNase Activity (16 Hour)** - A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and a minimum of 100 units of EcoRI-HF® 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)** - EcoRI-HF® is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.
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<tr>
<th>Assay Name/Specification (minimum release criteria)</th>
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<tbody>
<tr>
<td><strong>qPCR DNA Contamination (E. coli Genomic)</strong> - A minimum of 20 units of EcoRI-HF® is screened for the presence of <em>E. coli</em> genomic DNA using SYBR® Green qPCR with primers specific for the <em>E. coli</em> 16S rRNA locus. Results are quantified using a standard curve generated from purified <em>E. coli</em> genomic DNA. The measured level of <em>E. coli</em> genomic DNA contamination is ≤ 1 <em>E. coli</em> genome.</td>
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<td><strong>RNase Activity (Extended Digestion)</strong> - A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 20 units of EcoRI-HF® is incubated at 37°C. After incubation for 4 hours, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</td>
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Nancy Considine  
Quality Approver  

Date 07 Nov 2023