Product Name: EcoRI-HF®
Catalog Number: R3101S
Concentration: 20,000 U/ml
Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA in 1 hour at 37°C in a total reaction volume of 50 µl.
Lot Number: 10032169
Expiration Date: 09/2020
Storage Temperature: -20°C
Storage Conditions: 300 mM NaCl, 10 mM KPO4, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, 0.15 % TritonX-100, 200 µg/ml BSA, (pH 7.0 @ 25°C)
Specification Version: PS-R3101S/L v2.0

<table>
<thead>
<tr>
<th>NEB Part Number</th>
<th>Component Description</th>
<th>Lot Number</th>
<th>Individual QC Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>R3101SVIAL</td>
<td>EcoRI-HF®</td>
<td>10022888</td>
<td>Pass</td>
</tr>
<tr>
<td>B7204SVIAL</td>
<td>CutSmart® Buffer</td>
<td>10031557</td>
<td>Pass</td>
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<tr>
<td>B7024SVIAL</td>
<td>Gel Loading Dye, Purple (6X)</td>
<td>10021135</td>
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</tbody>
</table>

Assay Name/Specification

**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 CutSmart™ 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 CutSmart™ 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
<table>
<thead>
<tr>
<th>Assay Name/Specification</th>
<th>Lot # 10032169</th>
</tr>
</thead>
<tbody>
<tr>
<td>fragments, &gt;95% can be recut with EcoRI-HF™.</td>
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</tbody>
</table>

**Non-Specific DNase Activity (16 Hour)**
A 50 µl reaction in CutSmart™ 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.

This product has been tested and shown to be in compliance with all specifications.

Stephanie Cornelio  
Production Scientist  
25 Sep 2018

Mary Conlon  
Packaging Quality Control Inspector  
26 Dec 2018