EcoRI-HF® Component List

<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>R3101MVIAL</td>
<td>EcoRI-HF®</td>
<td>10035420</td>
<td>Pass</td>
</tr>
<tr>
<td>B7204SVIAL</td>
<td>CutSmart® Buffer</td>
<td>10031567</td>
<td>Pass</td>
</tr>
<tr>
<td>B7024SVIAL</td>
<td>Gel Loading Dye, Purple (6X)</td>
<td>10021141</td>
<td>Pass</td>
</tr>
</tbody>
</table>

Assay Name/Specification

<table>
<thead>
<tr>
<th>Lot # 10035419</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ligation and Recutting (Terminal Integrity)</strong>&lt;br&gt;After a 20-fold over-digestion of Lambda DNA with EcoRI-HF™, &gt;95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, &gt;95% can be recut with EcoRI-HF™.</td>
</tr>
<tr>
<td><strong>Non-Specific DNase Activity (16 Hour)</strong>&lt;br&gt;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.</td>
</tr>
<tr>
<td><strong>Protein Purity Assay (SDS-PAGE)</strong>&lt;br&gt;EcoRI-HF™ is &gt;95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.</td>
</tr>
<tr>
<td><strong>Blue-White Screening (Terminal Integrity)</strong>&lt;br&gt;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 &lt;1% white colonies.</td>
</tr>
<tr>
<td>Assay Name/Specification</td>
</tr>
<tr>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td><strong>Endonuclease Activity (Nicking)</strong></td>
</tr>
<tr>
<td>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 37ºC results in &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</td>
</tr>
<tr>
<td><strong>Exonuclease Activity (Radioactivity Release)</strong></td>
</tr>
<tr>
<td>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 37ºC releases &lt;0.1% of the total radioactivity.</td>
</tr>
</tbody>
</table>

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

Tony Spear-Alfonso  
Production Scientist  
16 Jan 2019

Michael Tonello  
Packaging Quality Control Inspector  
27 Feb 2019