New England Biolabs
Certificate of Analysis

Product Name: EcoRI-HF®
Catalog #: R3101S/L
Concentration: 20,000 units/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 #: 0131609
Assay Date: 09/2016
Expiration Date: 9/2018
Storage Temp: -20°C
Storage Conditions: 300 mM NaCl, 10 mM KPO$_4$, 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
Effective Date: 16 May 2016

<table>
<thead>
<tr>
<th>Assay Name/Specification (minimum release criteria)</th>
<th>Lot #0131609</th>
</tr>
</thead>
<tbody>
<tr>
<td>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 &lt;1% white colonies.</td>
<td>Pass</td>
</tr>
<tr>
<td>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 &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</td>
<td>Pass</td>
</tr>
<tr>
<td>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 &lt;0.1% of the total radioactivity.</td>
<td>Pass</td>
</tr>
<tr>
<td>Ligation and Recutting (Terminal Integrity) - 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>
<td>Pass</td>
</tr>
<tr>
<td>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.</td>
<td>Pass</td>
</tr>
<tr>
<td>Protein Purity Assay (SDS-PAGE) - EcoRI-HF™ is &gt;95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.</td>
<td>Pass</td>
</tr>
</tbody>
</table>

R3101S/L Lot: 0131609
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* The BSA in this product has been granted an EDQM "Certificate of Suitability" from the European Directorate for the Quality of Medicines (R1-CEP-2003-204-Rev00) and has been granted a USDA Certificate for Export of Bovine Blood Plasma/Serum for Manufacture into Pharmaceutical Products.

Authorized by
Derek Robinson
16 May 2016

Inspected by
Stephanie Cornelio
05 Aug 2016