New England Biolabs
Product Specification

Product Name: NotI-HF®
Catalog #: R3189S/L
Concentration: 20,000 units/ml
Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of pBC4 DNA in 1 hour at 37°C in a total reaction volume of 50 µl.
Shelf Life: 24 months
Storage Temp: -20 °C
Storage Conditions: 50 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml BSA
Specification Version: PS-R3189S/L v1.0
Effective Date: 03 May 2013

<table>
<thead>
<tr>
<th>Assay Name/Specification</th>
<th>(minimum release criteria)</th>
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<tbody>
<tr>
<td>Endonuclease Activity (Nicking) -</td>
<td>A 50 µl reaction in CutSmart™ Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 100 Units of NotI-HF™ incubated for 4 hours at 37°C results in &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</td>
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<tr>
<td>Exonuclease Activity (Radioactivity Release) -</td>
<td>A 50 µl reaction in CutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [^3\text{H}]\ E. coli DNA and a minimum of 200 units of NotI-HF™ incubated for 4 hours at 37°C releases &lt;0.1% of the total radioactivity.</td>
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<td>Ligation and Recutting (Terminal Integrity) -</td>
<td>After a 10-fold over-digestion of pBC4 DNA with NotI-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 NotI-HF™.</td>
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<tr>
<td>Non-Specific DNase Activity (16 Hour) -</td>
<td>A 50 µl reaction in CutSmart™ Buffer containing 1 µg of pBC4 DNA and a minimum of 200 Units of NotI-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>
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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.

Derek Robinson
Director of Quality Control

Date 03 May 2013