New England Biolabs
Certificate of Analysis

Product Name: NotI
Catalog #: R0189M
Concentration: 50,000 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.
Lot #: 0551708
Assay Date: 08/2017
Expiration Date: 8/2019
Storage Temp: -20°C
Storage Conditions: 250 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 0.15% Triton X-100, 200 µg/ml BSA
Specification Version: PS-R0189M v1.0
Effective Date: 31 Jul 2013

<table>
<thead>
<tr>
<th>Assay Name/Specification (minimum release criteria)</th>
<th>Lot #0551708</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endonuclease Activity (Nicking)</strong> - A 50 µl reaction in NEBuffer 3.1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 100 Units of NotI 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><strong>Exonuclease Activity (Radioactivity Release)</strong> - A 50 µl reaction in NEBuffer 3.1 containing 1 µg of a mixture of single and double-stranded [³H] E. coli DNA and a minimum of 100 units of NotI incubated for 4 hours at 37°C releases &lt;0.1% of the total radioactivity.</td>
<td>Pass</td>
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<tr>
<td><strong>Ligation and Recutting (Terminal Integrity)</strong> - After a 10-fold over-digestion of pBC4 DNA with NotI, &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.</td>
<td>Pass</td>
</tr>
<tr>
<td><strong>Non-Specific DNase Activity (16 Hour)</strong> - A 50 µl reaction in NEBuffer 3.1 containing 1 µg of pBC4 DNA and a minimum of 100 Units of NotI 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>
</tbody>
</table>

* 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
31 Jul 2013

Inspected by
Kerri Sakowski
29 Aug 2017

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