New England Biolabs  
Certificate of Analysis

**Product Name:**  
AscI

**Catalog #:**  
R0558S/L

**Concentration:**  
10,000 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 #:**  
0121409

**Assay Date:**  
09/2014

**Expiration Date:**  
9/2016

**Storage Temp:**  
-20 ºC

**Storage Conditions:**  
50 mM KCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml BSA

**Specification Version:**  
PS-R0558S/L v1.0

**Effective Date:**  
24 May 2013

<table>
<thead>
<tr>
<th>Assay Name/Specification (minimum release criteria)</th>
<th>Lot #0121409</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blue-White Screening (Terminal Integrity)</strong> - A sample of pNEB193 vector linearized with a 10-fold excess of AscI, 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><strong>Endonuclease Activity (Nicking)</strong> - A 50 µl reaction in CutSmart™ Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 30 Units of AscI incubated for 4 hours at 37ºC 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 CutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [³H] E. coli DNA and a minimum of 100 units of AscI incubated for 4 hours at 37ºC releases &lt;0.1% of the total radioactivity.</td>
<td>Pass</td>
</tr>
<tr>
<td><strong>Ligation and Recutting (Terminal Integrity)</strong> - After a 10-fold over-digestion of Lambda DNA with AscI, &gt;95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16ºC. Of these ligated fragments, &gt;95% can be recut with AscI.</td>
<td>Pass</td>
</tr>
<tr>
<td><strong>Non-Specific DNase Activity (16 Hour)</strong> - A 50 µl reaction in CutSmart™ Buffer containing 1 µg of Lambda DNA and a minimum of 30 Units of AscI incubated for 16 hours at 37ºC results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.</td>
<td>Pass</td>
</tr>
</tbody>
</table>
New England Biolabs
Certificate of Analysis

* 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
24 May 2013

Inspected by
Anthony Francis
17 Oct 2014