New England Biolabs
Certificate of Analysis

Product Name: Ascl
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 #: 0131708
Assay Date: 08/2017
Expiration Date: 08/2019
Storage Temp: -20°C
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: 19 Jul 2017

<table>
<thead>
<tr>
<th>Assay Name/Specification (minimum release criteria)</th>
<th>Lot #0131708</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue-White Screening (Terminal Integrity) - A sample of pNEB193 vector linearized with a 10-fold excess of Ascl, 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 30 Units of Ascl 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 100 units of Ascl 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 10-fold over-digestion of Lambda DNA with Ascl, &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 Ascl.</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 30 Units of Ascl 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.
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Authorized by
Derek Robinson
19 Jul 2017

Inspected by
Anthony Francis
28 Jul 2017