New England Biolabs  
Certificate of Analysis

Product Name: SphI  
Catalog #: R0182M  
Concentration: 80,000 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 #: 0481409  
Assay Date: 09/2014  
Expiration Date: 9/2016  
Storage Temp: -20 °C  
Storage Conditions: 100 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml BSA

Assay Name/Specification (minimum release criteria)  
Lot #0481409

<table>
<thead>
<tr>
<th>Assay Name/Specification</th>
<th>Lot #0481409</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue-White Screening (Terminal Integrity) - A sample of pUC19 vector linearized with a 10-fold excess of SphI, 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 NEBuffer 2.1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 30 Units of SphI incubated for 4 hours at 37°C results in &lt;20% 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 NEBuffer 2.1 containing 1 µg of a mixture of single and double-stranded [³H] E. coli DNA and a minimum of 100 units of SphI 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 SphI, &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 SphI.</td>
<td>Pass</td>
</tr>
<tr>
<td>Non-Specific DNase Activity (16 hour) - A 50 µl reaction in NEBuffer 2.1 containing 1 µg of Lambda DNA and a minimum of 10 Units of SphI incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis. NOTE: although no nuclease degradation is detected under these conditions, extended incubations and/or high concentrations of this enzyme may result in star activity. See the product FAQ for recommended reaction conditions for this enzyme.</td>
<td>Pass</td>
</tr>
<tr>
<td>Protein Purity Assay (SDS-PAGE) - SphI is &gt;95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.</td>
<td>Pass</td>
</tr>
</tbody>
</table>
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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
02 Aug 2013

Inspected by
Mike Dalton
26 Sep 2014