**New England Biolabs**  
**Certificate of Analysis**

**Product Name:** SpeI  
**Catalog #:** R0133S/L  
**Concentration:** 10,000 units/ml  
**Unit Definition:** One unit is defined as the amount of enzyme required to digest 1 µg of pXba-XbaI DNA in 1 hour at 37°C in a total reaction volume of 50 µl.

**Lot #:** 0321305  
**Assay Date:** 05/2013  
**Expiration Date:** 05/2015  
**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-R0133S/L v1.0  
**Effective Date:** 07 Jun 2013

<table>
<thead>
<tr>
<th>Assay Name/Specification (minimum release criteria)</th>
<th>Lot #0321305</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blue-White Screening (Terminal Integrity)</strong> - A sample of LITMUS28 vector linearized with a 10-fold excess of SpeI, 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 50 Units of SpeI 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><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 50 units of SpeI incubated for 4 hours at 37°C releases &lt;0.1% of the total radioactivity.</td>
<td>Pass</td>
</tr>
<tr>
<td><strong>Ligation and Recutting (Terminal Integrity)</strong> - After a 20-fold over-digestion of Adenovirus-2 DNA with SpeI, &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 SpeI.</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 pXba-XbaI digested DNA and a minimum of 50 units of SpeI 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>
<tr>
<td><strong>Protein Purity Assay (SDS-PAGE)</strong> - SpeI 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
07 Jun 2013

Inspected by
Terry Petronzio
07 Aug 2013