

## New England Biolabs Certificate of Analysis

**Product Name:** *SpeI*  
**Catalog Number:** *R0133S*  
**Concentration:** *10,000 U/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.*  
**Packaging Lot Number:** *10125121*  
**Expiration Date:** *03/2023*  
**Storage Temperature:** *-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 v2.0*

Spel Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R0133SVIAL	Spel	10102168	Pass
B7024AVIAL	Gel Loading Dye, Purple (6X)	10121396	Pass
B6004SVIAL	rCutSmart™ Buffer	10127377	Pass

Assay Name/Specification	Lot # 10125121
<p><b>Non-Specific DNase Activity (16 Hour)</b>            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.</p>	Pass
<p><b>Ligation and Recutting (Terminal Integrity)</b>            After a 20-fold over-digestion of T7 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.</p>	Pass
<p><b>Blue-White Screening (Terminal Integrity)</b>            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.</p>	Pass
<p><b>Endonuclease Activity (Nicking)</b></p>	Pass

Assay Name/Specification	Lot # 10125121
<p>A 50 µl reaction in CutSmart® Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 50 units of Spel incubated for 4 hours at 37°C results in &lt;20% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	
<p><b>Exonuclease Activity (Radioactivity Release)</b> A 50 µl reaction in CutSmart® Buffer containing 1 µg of a mixture of single and double-stranded [<sup>3</sup>H] E. coli DNA and a minimum of 50 units of Spel incubated for 4 hours at 37°C releases &lt;0.1% of the total radioactivity.</p>	<b>Pass</b>
<p><b>Protein Purity Assay (SDS-PAGE)</b> Spel is &gt;95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>

This product has been tested and shown to be in compliance with all specifications.

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