

## New England Biolabs Certificate of Analysis

**Product Name:** Spel-HF<sup>®</sup>  
**Catalog Number:** R3133S  
**Concentration:** 20,000 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:** 10152191  
**Expiration Date:** 04/2024  
**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<sup>®</sup> X-100, 200 µg/ml BSA  
**Specification Version:** PS-R3133S/L v2.0

Spel-HF <sup>®</sup> Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R3133SVIAL	Spel-HF <sup>®</sup>	10147569	Pass
B7024AVIAL	Gel Loading Dye, Purple (6X)	10150372	Pass
B6004SVIAL	rCutSmart <sup>™</sup> Buffer	10150373	Pass

Assay Name/Specification	Lot # 10152191
<b>Blue-White Screening (Terminal Integrity)</b> A sample of LITMUS28 vector linearized with a 10-fold excess of Spel-HF <sup>®</sup> , religated and transformed into an E. coli strain expressing the LacZ beta fragment gene results in <1% white colonies.	Pass
<b>Endonuclease Activity (Nicking)</b> A 50 µl reaction in CutSmart <sup>®</sup> Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 60 units of Spel-HF <sup>®</sup> incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
<b>Non-Specific DNase Activity (16 Hour)</b> A 50 µl reaction in CutSmart <sup>®</sup> Buffer containing 1 µg of pXba-XbaI digested DNA and a minimum of 100 units of Spel-HF <sup>®</sup> incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	Pass
<b>Ligation and Recutting (Terminal Integrity)</b>	Pass

Assay Name/Specification	Lot # 10152191
<p>After a 20-fold over-digestion of T7 DNA with Spel-HF<sup>®</sup>, &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 Spel-HF<sup>®</sup>.</p>	
<p><b>Exonuclease Activity (Radioactivity Release)</b> A 50 µl reaction in CutSmart<sup>®</sup> Buffer containing 1 µg of a mixture of single and double-stranded [<sup>3</sup>H] E. coli DNA and a minimum of 100 units of Spel-HF<sup>®</sup> 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-HF<sup>®</sup> is ≥ 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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07 Jun 2022



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