

## New England Biolabs Certificate of Analysis

**Product Name:** Spel-HF®  
**Catalog Number:** R3133M  
**Concentration:** 100,000 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.  
**Lot Number:** 10015015  
**Expiration Date:** 07/2020  
**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-R3133M v2.0

Spel-HF® Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R3133M VIAL	Spel-HF®	10015016	Pass
B7204S VIAL	CutSmart® Buffer	10010634	Pass
B7024S VIAL	Gel Loading Dye, Purple (6X)	0261805	Pass

Assay Name/Specification	Lot # 10015015
<b>Blue-White Screening (Terminal Integrity)</b> A sample of LITMUS28 vector linearized with a 10-fold excess of Spel-HF®, 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® Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 60 units of Spel-HF® incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
<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 100 units of Spel-HF® incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
<b>Ligation and Recutting (Terminal Integrity)</b> After a 20-fold over-digestion of T7 DNA with Spel-HF®, >95% of the DNA fragments	Pass

Assay Name/Specification	Lot # 10015015
<p>can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, &gt;95% can be recut with SpeI-HF®.</p> <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 100 units of SpeI-HF® incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.</p> <p><b>Protein Purity Assay (SDS-PAGE)</b> SpeI-HF® is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<p style="text-align: center;"><b>Pass</b></p> <p style="text-align: center;"><b>Pass</b></p>

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



Tony Spear-Alfonso  
Production Scientist  
13 Jun 2018



Michael Tonello  
Packaging Quality Control Inspector  
19 Jul 2018