

## New England Biolabs Certificate of Analysis

**Product Name:** SphI-HF®  
**Catalog Number:** R3182S  
**Concentration:** 20,000 U/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.  
**Packaging Lot Number:** 10138393  
**Expiration Date:** 11/2023  
**Storage Temperature:** -20°C  
**Storage Conditions:** 200 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml BSA  
**Specification Version:** PS-R3182S/L v1.0

SphI-HF® Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R3182SVIAL	SphI-HF®	10127884	Pass
B7024AVIAL	Gel Loading Dye, Purple (6X)	10130600	Pass
B6004SVIAL	rCutSmart™ Buffer	10138402	Pass

Assay Name/Specification	Lot # 10138393
<p><b>Non-Specific DNase Activity (16 Hour)</b>            A 50 µl reaction in CutSmart™ Buffer containing 1 µg of Lambda DNA and a minimum of 100 units of SphI-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>	Pass
<p><b>Ligation and Recutting (Terminal Integrity)</b>            After a 20-fold over-digestion of Lambda DNA with SphI-HF™, &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-HF™.</p>	Pass
<p><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 SphI-HF™ incubated for 4 hours at 37°C results in &lt;20% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	Pass
<p><b>Blue-White Screening (Terminal Integrity)</b>            A sample of pUC19 vector linearized with a 10-fold excess of SphI-HF™, religated and transformed into an E. coli strain expressing the LacZ beta fragment gene</p>	Pass

Assay Name/Specification	Lot # 10138393
results in <1% white colonies.	
<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 100 units of SphI-HF™ 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> SphI-HF™ 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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11 Mar 2022



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