

## New England Biolabs Certificate of Analysis

*Product Name:* SphI-HF<sup>®</sup>  
*Catalog #:* R3182M  
*Concentration:* 100,000 units/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.  
*Lot #:* 0031706  
*Assay Date:* 06/2017  
*Expiration Date:* 6/2019  
*Storage Temp:* -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-R3182M v1.0  
*Effective Date:* 30 Apr 2015

Assay Name/Specification (minimum release criteria)	Lot #0031706
<b>Blue-White Screening (Terminal Integrity)</b> - A sample of pUC19 vector linearized with a 10-fold excess of SphI-HF <sup>™</sup> , religated and transformed into an <i>E. coli</i> strain expressing the LacZ beta fragment gene results in <1% white colonies.	<b>Pass</b>
<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 SphI-HF <sup>™</sup> incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.	<b>Pass</b>
<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] <i>E. coli</i> DNA and a minimum of 100 units of SphI-HF <sup>™</sup> incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	<b>Pass</b>
<b>Ligation and Recutting (Terminal Integrity)</b> - After a 20-fold over-digestion of Lambda DNA with SphI-HF <sup>™</sup> , >95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, >95% can be recut with SphI-HF <sup>™</sup> .	<b>Pass</b>
<b>Non-Specific DNase Activity (16 Hour)</b> - A 50 µl reaction in CutSmart <sup>™</sup> Buffer containing 1 µg of Lambda DNA and a minimum of 100 units of SphI-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.	<b>Pass</b>
<b>Protein Purity Assay (SDS-PAGE)</b> - SphI-HF <sup>™</sup> is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.	<b>Pass</b>



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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.



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Authorized by  
Derek Robinson  
30 Apr 2015



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Inspected by  
Penghua Zhang  
14 Jun 2017

