

New England Biolabs Certificate of Analysis

Product Name: SphI-HF®
Catalog Number: R3182M
Concentration: 100,000 U/ml
Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA in rCutSmart Buffer in 1 hour at 37°C in a total reaction volume of 50 µl.
Packaging Lot Number: 10213823
Expiration Date: 09/2025
Storage Temperature: -20°C
Storage Conditions: 10 mM Tris-HCl, 200 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml rAlbumin (pH 7.4 @ 25°C)
Specification Version: PS-R3182M v2.0

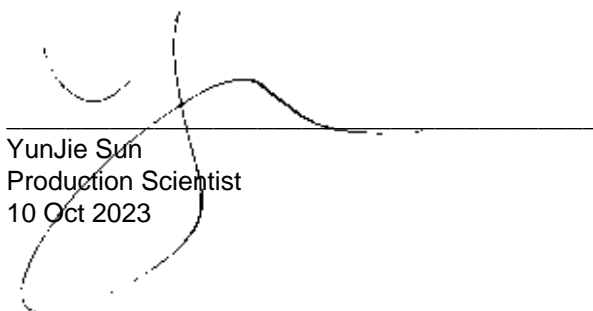
SphI-HF® Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R3182M VIAL	SphI-HF®	10209083	Pass
B7024A VIAL	Gel Loading Dye, Purple (6X)	10202501	Pass
B6004S VIAL	rCutSmart™ Buffer	10204840	Pass

Assay Name/Specification	Lot # 10213823
Blue-White Screening (Terminal Integrity) 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 results in <1% white colonies.	Pass
Endonuclease Activity (Nicking) A 50 µl reaction in rCutSmart™ 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 <20% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
Exonuclease Activity (Radioactivity Release) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [³ H] E. coli DNA and a minimum of 100 units of SphI-HF® incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
Functional Testing (15 minute Digest) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and 1 µl of	Pass

Assay Name/Specification	Lot # 10213823
<p>SphI-HF[®] incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.</p>	
<p>Ligation and Recutting (Terminal Integrity) After a 20-fold over-digestion of Lambda DNA with SphI-HF[®], >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[®].</p>	Pass
<p>Non-Specific DNase Activity (16 Hour) A 50 µl reaction in rCutSmart[™] 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>Protein Purity Assay (SDS-PAGE) SphI-HF[®] is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.</p>	Pass
<p>qPCR DNA Contamination (E. coli Genomic) A minimum of 20 units of SphI-HF[®] is screened for the presence of E. coli genomic DNA using SYBR[®] Green qPCR with primers specific for the E. coli 16S rRNA locus. Results are quantified using a standard curve generated from purified E. coli genomic DNA. The measured level of E. coli genomic DNA contamination is ≤ 1 E. coli genome.</p>	Pass

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

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.



YunJie Sun
Production Scientist
10 Oct 2023



Michael Tonello
Packaging Quality Control Inspector
02 Nov 2023