

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 rCutSmart Buffer in 1 hour at 37°C in a total reaction volume of 50 µl.
Packaging Lot Number: 10192272
Expiration Date: 10/2024
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-R3182S/L v2.0

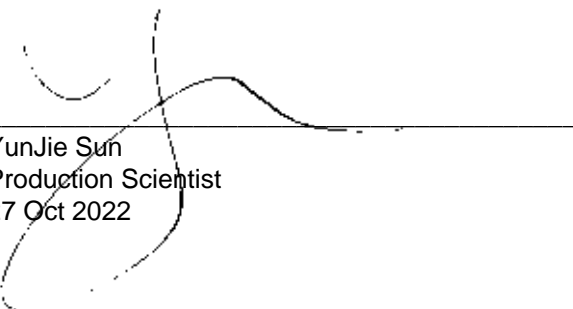
SphI-HF [®] Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R3182SVIAL	SphI-HF [®]	10168207	Pass
B7024AVIAL	Gel Loading Dye, Purple (6X)	10189226	Pass
B6004SVIAL	rCutSmart [™] Buffer	10189224	Pass

Assay Name/Specification	Lot # 10192272
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 # 10192272
SphI-HF® incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.	
<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.



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Production Scientist
27 Oct 2022



Josh Hersey
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28 Jun 2023