

New England Biolabs Certificate of Analysis

Product Name: SphI-HF[®]
Catalog Number: R3182L
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: 10091405
Expiration Date: 11/2022
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
R3182LVIAL	SphI-HF [®]	10091400	Pass
B7204SVIAL	CutSmart [®] Buffer	10091458	Pass
B7024AVIAL	Gel Loading Dye, Purple (6X)	10089393	Pass

Assay Name/Specification	Lot # 10091405
Endonuclease Activity (Nicking) 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 <20% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
Exonuclease Activity (Radioactivity Release) A 50 µl reaction in CutSmart [™] 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
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 [™] .	Pass
Non-Specific DNase Activity (16 Hour) 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	Pass

Assay Name/Specification	Lot # 10091405
of detectable nuclease degradation as determined by agarose gel electrophoresis.	
Protein Purity Assay (SDS-PAGE) SphI-HF™ is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.	Pass
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

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.



Penghua Zhang
Production Scientist
08 Jan 2021



Michael Tonello
Packaging Quality Control Inspector
08 Jan 2021