

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: 10068132
Expiration Date: 09/2021
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 [®]	10054651	Pass
B7204SVIAL	CutSmart [®] Buffer	10069109	Pass
B7024SVIAL	Gel Loading Dye, Purple (6X)	10065745	Pass

Assay Name/Specification	Lot # 10068132
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 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	Pass

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fragments, >95% can be recut with SphI-HF™.	
<p>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 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

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



Anthony Francis
Production Scientist
26 Sep 2019



Jay Minichiello
Packaging Quality Control Inspector
27 Mar 2020