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New England Biolabs Certificate of Analysis

Product Name: Sspl-HF®
Catalog Number: R3132M
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: 10175010
Expiration Date: 12/2024
Storage Temperature: -20°C

Storage Conditions: 10 mM Tris-HCl, 200 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol,

200 $\mu g/ml$ rAlbumin (pH 7.4 @ 25°C)

Specification Version: PS-R3132M v2.0

SspI-HF® Component List				
NEB Part Number	Component Description	Lot Number	Individual QC Result	
R3132MVIAL	SspI-HF®	10175009	Pass	
B7024AVIAL	Gel Loading Dye, Purple (6X)	10163561	Pass	
B6004SVIAL	rCutSmart™ Buffer	10173161	Pass	

Assay Name/Specification	Lot # 10175010
Protein Purity Assay (SDS-PAGE) SspI-HF® is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
qPCR DNA Contamination (E. coli Genomic) A minimum of 20 units of SspI-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.	Pass
Exonuclease Activity (Radioactivity Release) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [³H] E. coli DNA and a minimum of 100 units of Sspl-HF® incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
Ligation and Recutting (Terminal Integrity)	Pass



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After a 10-fold over-digestion of Lambda DNA with SspI-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 SspI-HF®.	
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 Sspl-HF® incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	Pass
Functional Testing (15 minute Digest) A 50 μl reaction in rCutSmart™ Buffer containing 1 μg of Lambda DNA and 1 μl of Sspl-HF® incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.	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 V Production Scientist 14 Dec 2022 Michael Tonello

Packaging Quality Control Inspector

15 Dec 2022



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