

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: 10264943
Expiration Date: 09/2026
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® | 10256951 | Pass |
| B7024AVIAL | Gel Loading Dye, Purple (6X) | 10250204 | Pass |
| B6004SVIAL | rCutSmart™ Buffer | 10256011 | Pass |

| Assay Name/Specification | Lot # 10264943 |
|---|----------------|
| 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 |

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|---|----------------|
| <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.



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29 Oct 2024



Michael Tonello
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06 Nov 2024