

## 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:** 10118231  
**Expiration Date:** 05/2023  
**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®                     | 10107065   | Pass                 |
| B7024AVIAL              | Gel Loading Dye, Purple (6X) | 10105819   | Pass                 |
| B6004SVIAL              | rCutSmart™ Buffer            | 10111605   | Pass                 |

| Assay Name/Specification  | Lot # 10118231 |
|---|----------------|
| <b>Protein Purity Assay (SDS-PAGE)</b><br>SphI-HF™ is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.  | Pass           |
| <b>Ligation and Recutting (Terminal Integrity)</b><br>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           |
| <b>Non-Specific DNase Activity (16 Hour)</b><br>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. | Pass           |
| <b>Exonuclease Activity (Radioactivity Release)</b><br>A 50 µl reaction in CutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [ <sup>3</sup> 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           |

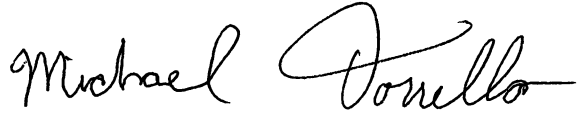
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|--|----------------|
| <p><b>Blue-White Screening (Terminal Integrity)</b><br/>A sample of pUC19 vector linearized with a 10-fold excess of SphI-HF™, religated and transformed into an E. coli strain expressing the LacZ beta fragment gene results in &lt;1% white colonies.</p>   | <b>Pass</b>    |
| <p><b>Endonuclease Activity (Nicking)</b><br/>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 &lt;20% conversion to the nicked form as determined by agarose gel electrophoresis.</p> | <b>Pass</b>    |

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

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10 Aug 2021



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