

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.
Lot Number: 10033681
Expiration Date: 11/2020
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® | 10027910 | Pass |
| B7204SVIAL | CutSmart® Buffer | 10036664 | Pass |
| B7024SVIAL | Gel Loading Dye, Purple (6X) | 10021142 | Pass |

| Assay Name/Specification | Lot # 10033681 |
|--|----------------|
| 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
20 Nov 2018



Michael Tonello
Packaging Quality Control Inspector
18 Mar 2019