

New England Biolabs Certificate of Analysis

Product Name: SphI-HF[®]
Catalog #: R3182M
Concentration: 100,000 units/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 #: 0011212
Assay Date: 12/2012
Expiration Date: 12/2014
Storage Temp: -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-R3182M v1.0
Effective Date: 02 Aug 2013

| Assay Name/Specification (minimum release criteria) | Lot #0011212 |
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| Blue-White Screening (Terminal Integrity) - A sample of pUC19 vector linearized with a 10-fold excess of SphI-HF [™] , religated and transformed into an <i>E. coli</i> 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] <i>E. coli</i> 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 fragments, >95% can be recut with SphI-HF [™] . | Pass |
| 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. | Pass |
| Protein Purity Assay (SDS-PAGE) - SphI-HF [™] is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection. | Pass |

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* The BSA in this product has been granted an EDQM "Certificate of Suitability" from the European Directorate for the Quality of Medicines (# R1-CEP-2003-204-Rev00) and has been granted a USDA Certificate for Export of Bovine Blood Plasma/Serum for Manufacture into Pharmaceutical Products.



Authorized by
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02 Aug 2013



Inspected by
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02 Aug 2013

