

## New England Biolabs Product Specification

<i>Product Name:</i>	<i>SphI-HF<sup>®</sup></i>
<i>Catalog #:</i>	<i>R3182M</i>
<i>Concentration:</i>	<i>100,000 units/ml</i>
<i>Unit Definition:</i>	<i>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.</i>
<i>Shelf Life:</i>	<i>24 months</i>
<i>Storage Temp:</i>	<i>-20 °C</i>
<i>Storage Conditions:</i>	<i>200 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml BSA</i>
<i>Specification Version:</i>	<i>PS-R3182M v1.0</i>
<i>Effective Date:</i>	<i>02 Aug 2013</i>

### Assay Name/Specification (minimum release criteria)

**Blue-White Screening (Terminal Integrity)** - A sample of pUC19 vector linearized with a 10-fold excess of SphI-HF<sup>™</sup>, religated and transformed into an *E. coli* strain expressing the LacZ beta fragment gene results in <1% white colonies.

**Endonuclease Activity (Nicking)** - A 50 µl reaction in CutSmart<sup>™</sup> Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 60 units of SphI-HF<sup>™</sup> incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.

**Exonuclease Activity (Radioactivity Release)** - A 50 µl reaction in CutSmart<sup>™</sup> 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<sup>™</sup> incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

**Ligation and Recutting (Terminal Integrity)** - After a 20-fold over-digestion of Lambda DNA with SphI-HF<sup>™</sup>, >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<sup>™</sup>.

**Non-Specific DNase Activity (16 Hour)** - A 50 µl reaction in CutSmart<sup>™</sup> Buffer containing 1 µg of Lambda DNA and a minimum of 100 units of SphI-HF<sup>™</sup> incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

**Protein Purity Assay (SDS-PAGE)** - SphI-HF<sup>™</sup> is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.

\* 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.



Date 02 Aug 2013

Derek Robinson

Director of Quality Control

