

## New England Biolabs Product Specification

<b>Product Name:</b>	SacI-HF <sup>®</sup>
<b>Catalog #:</b>	R3156S/L
<b>Concentration:</b>	20,000 units/ml
<b>Unit Definition:</b>	One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA (HindIII digest) in 1 hour at 37°C in a total reaction volume of 50 µl.
<b>Shelf Life:</b>	24 months
<b>Storage Temp:</b>	-20°C
<b>Storage Conditions:</b>	100 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml BSA
<b>Specification Version:</b>	PS-R3156S/L v2.0
<b>Effective Date:</b>	04 Dec 2017

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

**Blue-White Screening (Terminal Integrity)** - A sample of LITMUS28i vector linearized with a 10-fold excess of SacI-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 SacI-HF<sup>™</sup> incubated for 4 hours at 37°C results in <10% 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 SacI-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 pXba DNA with SacI-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 SacI-HF<sup>™</sup>.

**Non-Specific DNase Activity (16 Hour)** - A 50 µl reaction in CutSmart<sup>™</sup> Buffer containing 1 µg of Lambda-HindIII DNA and a minimum of 100 Units of SacI-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)** - SacI-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 04 Dec 2017

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Director of Quality Control

