

240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

## New England Biolabs Certificate of Analysis

Product Name:	SacI-HF®
Catalog #:	R3156S/L
Concentration:	20,000 units/ml
Unit Definition:	One unit is defined as the amount of enzyme required to digest 1 $\mu$ g of Lambda DNA (HindIII digest) in 1 hour at 37°C in a total reaction volume of 50 $\mu$ l.
Lot #:	0011503
Assay Date:	03/2015
Expiration Date:	3/2017
Storage Temp:	-20°C
Storage Conditions:	100 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 $\mu$ g/ml BSA
Specification Version:	PS-R3156S/L v1.0
Effective Date:	22 Jul 2013

Assay Name/Specification (minimum release criteria)	Lot #0011503
<b>Blue-White Screening (Terminal Integrity)</b> - A sample of LITMUS28i vector linearized with a 10-fold excess of SacI-HF <sup>TM</sup> , religated and transformed into an <i>E. coli</i> strain expressing the LacZ beta fragment gene results in <1% white colonies.	Pass
<b>Endonuclease Activity (Nicking)</b> - A 50 $\mu$ l reaction in CutSmart <sup>TM</sup> Buffer containing 1 $\mu$ g of supercoiled PhiX174 DNA and a minimum of 60 Units of SacI-HF <sup>TM</sup> incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
<b>Exonuclease Activity (Radioactivity Release)</b> - A 50 $\mu$ l reaction in CutSmart <sup>TM</sup> Buffer containing 1 $\mu$ g of a mixture of single and double-stranded [ <sup>3</sup> H] <i>E. coli</i> DNA and a minimum of 100 units of SacI-HF <sup>TM</sup> incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
<b>Ligation and Recutting (Terminal Integrity)</b> - After a 20-fold over-digestion of Lambda-HindIII DNA with SacI-HF <sup>TM</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>TM</sup> .	Pass
<b>Non-Specific DNase Activity (16 Hour)</b> - A 50 µl reaction in CutSmart <sup>TM</sup> Buffer containing 1 µg of Lambda- HindIII DNA and a minimum of 100 Units of SacI-HF <sup>TM</sup> 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>Protein Purity Assay (SDS-PAGE)</b> - SacI-HF <sup>™</sup> is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.	Pass



R3156S/L Lot: 0011503 Page 1 of 2



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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.

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Authorized by Derek Robinson 22 Jul 2013



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Inspected by Anthony Francis 10 Apr 2015