NEW ENGLAND BioLabs Inc.
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240 County Road
Ipswich, MA 01938-2723
Tel 978-927-5054
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www.neb.com
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New England Biolabs
Certificate of Analysis

Product Name:	*SacI-HF®*
Catalog Number:	*R3156S*
Concentration:	*20,000 U/ml*
Unit Definition:	*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.*
Lot Number:	*10054990*
Expiration Date:	*04/2021*
Storage Temperature:	*-20°C*
Storage Conditions:	*100 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-R3156S/L v2.0*

SacI-HF® Component List			
NEB Part Number	**Component Description**	**Lot Number**	**Individual QC Result**
R3156SVIAL	SacI-HF®	10043047	**Pass**
B7204SVIAL	CutSmart® Buffer	10043350	**Pass**
B7024SVIAL	Gel Loading Dye, Purple (6X)	10050274	**Pass**

Assay Name/Specification	**Lot # 10054990**
Blue-White Screening (Terminal Integrity) A sample of LITMUS28i vector linearized with a 10-fold excess of SacI-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 SacI-HF™ incubated for 4 hours at 37ºC results in <10% 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 SacI-HF™ incubated for 4 hours at 37ºC releases <0.1% of the total radioactivity.	**Pass**
Ligation and Recutting (Terminal Integrity) After a 20-fold over-digestion of pXba DNA with SacI-HF™, >95% of the DNA fragments	**Pass**

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Assay Name/Specification	Lot # 10054990
can be ligated with T4 DNA ligase in 16 hours at 16ºC. Of these ligated fragments, >95% can be recut with SacI-HF™.	
Non-Specific DNase Activity (16 Hour) A 50 µl reaction in CutSmart™ Buffer containing 1 µg of Lambda-HindIII DNA and a minimum of 100 Units of SacI-HF™ incubated for 16 hours at 37ºC results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	**Pass**
Protein Purity Assay (SDS-PAGE) SacI-HF™ is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.	**Pass**

This product has been tested and shown to be in compliance with all specifications.

Anthony Francis
Production Scientist
23 Apr 2019

Jay Minichiello
Packaging Quality Control Inspector
30 Sep 2019