NEW ENGLAND BioLabs Inc.
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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 rCutSmart Buffer in 1 hour at 37°C in a total reaction volume of 50 µl.*
Packaging Lot Number:	*10205497*
Expiration Date:	*07/2025*
Storage Temperature:	*-20°C*
Storage Conditions:	*10 mM Tris-HCl, 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml rAlbumin (pH 7.4 @ 25°C)*
Specification Version:	*PS-R3156S/L v3.0*

SacI-HF® Component List			
NEB Part Number	**Component Description**	**Lot Number**	**Individual QC Result**
R3156SVIAL	SacI-HF®	10195586	**Pass**
B7024AVIAL	Gel Loading Dye, Purple (6X)	10198639	**Pass**
B6004SVIAL	rCutSmart™ Buffer	10198644	**Pass**

Assay Name/Specification	**Lot # 10205497**
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 rCutSmart™ Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 60 units of SacI-HF® incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	**Pass**
Exonuclease Activity (Radioactivity Release) A 50 µl reaction in rCutSmart™ 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 37°C releases <0.1% of the total radioactivity.	**Pass**
Functional Testing (15 minute Digest) A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda-HindIII DNA and 1 µl	**Pass**

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Assay Name/Specification	Lot # 10205497
of SacI-HF® incubated for 15 minutes at 37ºC results in complete digestion as determined by agarose gel electrophoresis.	
Ligation and Recutting (Terminal Integrity) After a 20-fold over-digestion of pXba DNA with SacI-HF®, >95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16ºC. Of these ligated fragments, >95% can be recut with SacI-HF®.	**Pass**
Non-Specific DNase Activity (16 Hour) A 50 µl reaction in rCutSmart™ 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**
qPCR DNA Contamination (E. coli Genomic) A minimum of 20 units of SacI-HF® is screened for the presence of E. coli genomic DNA using SYBR® Green qPCR with primers specific for the E. coli 16S rRNA locus. Results are quantified using a standard curve generated from purified E. coli genomic DNA. The measured level of E. coli genomic DNA contamination is ≤ 1 E. coli genome.	**Pass**

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

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.

YunJie Sun
Production Scientist
04 Jul 2023

Josh Hersey
Packaging Quality Control Inspector
01 Sep 2023