

## 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:** *10263866*  
**Expiration Date:** *08/2026*  
**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®	10249945	Pass
B7024AVIAL	Gel Loading Dye, Purple (6X)	10245419	Pass
B6004SVIAL	rCutSmart™ Buffer	10250206	Pass

Assay Name/Specification	Lot # 10263866
<b>Blue-White Screening (Terminal Integrity)</b> 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.	<b>Pass</b>
<b>Endonuclease Activity (Nicking)</b> 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.	<b>Pass</b>
<b>Exonuclease Activity (Radioactivity Release)</b> A 50 µl reaction in rCutSmart™ 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® incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	<b>Pass</b>
<b>Functional Testing (15 minute Digest)</b> A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda-HindIII DNA and 1 µl	<b>Pass</b>

Assay Name/Specification	Lot # 10263866
<p>of SacI-HF® incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.</p>	
<p><b>Ligation and Recutting (Terminal Integrity)</b> After a 20-fold over-digestion of pXba DNA with SacI-HF®, &gt;95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, &gt;95% can be recut with SacI-HF®.</p>	<b>Pass</b>
<p><b>Non-Specific DNase Activity (16 Hour)</b> 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 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.</p>	<b>Pass</b>
<p><b>Protein Purity Assay (SDS-PAGE)</b> SacI-HF® is &gt;95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>
<p><b>qPCR DNA Contamination (E. coli Genomic)</b> 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.</p>	<b>Pass</b>

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

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