

## New England Biolabs Certificate of Analysis

**Product Name:** *SacI-HF<sup>®</sup>*  
**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.*  
**Packaging Lot Number:** *10140527*  
**Expiration Date:** *02/2024*  
**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*

<b>SacI-HF<sup>®</sup> Component List</b>			
<b>NEB Part Number</b>	<b>Component Description</b>	<b>Lot Number</b>	<b>Individual QC Result</b>
R3156SVIAL	SacI-HF <sup>®</sup>	10140525	<b>Pass</b>
B7024AVIAL	Gel Loading Dye, Purple (6X)	10132772	<b>Pass</b>
B6004SVIAL	rCutSmart <sup>™</sup> Buffer	10143285	<b>Pass</b>

<b>Assay Name/Specification</b>	<b>Lot # 10140527</b>
<b>Protein Purity Assay (SDS-PAGE)</b> SacI-HF <sup>™</sup> is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.	<b>Pass</b>
<b>Blue-White Screening (Terminal Integrity)</b> 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.	<b>Pass</b>
<b>Endonuclease Activity (Nicking)</b> 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.	<b>Pass</b>
<b>Exonuclease Activity (Radioactivity Release)</b> 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	<b>Pass</b>

Assay Name/Specification	Lot # 10140527
for 4 hours at 37°C releases <0.1% of the total radioactivity.	
<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 CutSmart™ 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>

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](http://www.neb.com/trademarks) for additional information.



Penghua Zhang  
Production Scientist  
21 Mar 2022



Michael Tonello  
Packaging Quality Control Inspector  
21 Mar 2022