

## New England Biolabs Certificate of Analysis

**Product Name:** *SacI-HF<sup>®</sup>*  
**Catalog Number:** *R3156M*  
**Concentration:** *100,000 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:** *10096526*  
**Expiration Date:** *01/2023*  
**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-R3156M v2.0*

SacI-HF <sup>®</sup> Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R3156M VIAL	SacI-HF <sup>®</sup>	10096527	Pass
B7204S VIAL	CutSmart <sup>®</sup> Buffer	10093117	Pass
B7024A VIAL	Gel Loading Dye, Purple (6X)	10089405	Pass

Assay Name/Specification	Lot # 10096526
<b>Non-Specific DNase Activity (16 Hour)</b> A 50 µl reaction in CutSmart <sup>™</sup> Buffer containing 1 µg of Lambda-HindIII DNA and a minimum of 100 Units of SacI-HF <sup>™</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
<b>Ligation and Recutting (Terminal Integrity)</b> After a 20-fold over-digestion of pXba DNA with SacI-HF <sup>™</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>™</sup> .	Pass
<b>Blue-White Screening (Terminal Integrity)</b> A sample of LITMUS28i vector linearized with a 10-fold excess of SacI-HF <sup>™</sup> ,	Pass

Assay Name/Specification	Lot # 10096526
<p>religated and transformed into an E. coli strain expressing the LacZ beta fragment gene results in &lt;1% white colonies.</p> <p><b>Endonuclease Activity (Nicking)</b> 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 37°C results in &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p> <p><b>Exonuclease Activity (Radioactivity Release)</b> A 50 µl reaction in CutSmart™ 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 &lt;0.1% of the total radioactivity.</p>	<p style="text-align: center;"><b>Pass</b></p> <p style="text-align: center;"><b>Pass</b></p>

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

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Packaging Quality Control Inspector  
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