

## New England Biolabs Certificate of Analysis

**Product Name:** NheI-HF<sup>®</sup>  
**Catalog Number:** R3131L  
**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:** 10156291  
**Expiration Date:** 07/2024  
**Storage Temperature:** -20°C  
**Storage Conditions:** 250 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 0.15% Triton X-100, 200 µg/ml BSA  
**Specification Version:** PS-R3131S/L v1.0

NheI-HF <sup>®</sup> Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R3131LVIAL	NheI-HF <sup>®</sup>	10156287	Pass
B7024AVIAL	Gel Loading Dye, Purple (6X)	10156431	Pass
B6004SVIAL	rCutSmart <sup>™</sup> Buffer	10156428	Pass

Assay Name/Specification	Lot # 10156291
<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 200 Units of NheI-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>Ligation and Recutting (Terminal Integrity)</b> After a 100-fold over-digestion of Lambda HindIII DNA with NheI-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 NheI-HF <sup>™</sup> .	Pass
<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 100 Units of NheI-HF <sup>™</sup> incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
<b>Blue-White Screening (Terminal Integrity)</b>	Pass

Assay Name/Specification	Lot # 10156291
<p>A sample of LITMUS28i vector linearized with a 10-fold excess of NheI-HF™, religated and transformed into an E. coli strain expressing the LacZ beta fragment gene results in &lt;1% white colonies.</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 300 units of NheI-HF™ incubated for 4 hours at 37°C releases &lt;0.1% of the total radioactivity.</p>	<p><b>Pass</b></p>

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

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12 Aug 2022