

## 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:** 10163496  
**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)  | 10158559   | Pass                 |
| B6004SVIAL                          | rCutSmart <sup>™</sup> Buffer | 10158558   | Pass                 |

| Assay Name/Specification   | Lot # 10163496 |
|--|----------------|
| <p><b>Non-Specific DNase Activity (16 Hour)</b><br/>           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.</p> | Pass           |
| <p><b>Ligation and Recutting (Terminal Integrity)</b><br/>           After a 100-fold over-digestion of Lambda HindIII DNA with NheI-HF<sup>™</sup>, &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 NheI-HF<sup>™</sup>.</p>                                  | Pass           |
| <p><b>Endonuclease Activity (Nicking)</b><br/>           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 &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p>                   | Pass           |
| <p><b>Blue-White Screening (Terminal Integrity)</b></p>  | Pass           |

| Assay Name/Specification  | Lot # 10163496     |
|---|--------------------|
| <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><br/>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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08 Aug 2022



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05 Oct 2022