

New England Biolabs Product Specification

Product Name:	<i>NheI-HF</i> [®]
Catalog #:	<i>R3131M</i>
Concentration:	<i>100,000 units/ml</i>
Unit Definition:	<i>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.</i>
Shelf Life:	<i>24 months</i>
Storage Temp:	<i>-20°C</i>
Storage Conditions:	<i>250 mM NaCl, 10 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 0.15% Triton X-100, 200 µg/ml rAlbumin (pH 7.4 @ 25°C)</i>
Specification Version:	<i>PS-R3131M v2.0</i>
Effective Date:	<i>07 Nov 2023</i>

Assay Name/Specification (minimum release criteria)

Blue-White Screening (Terminal Integrity) - A sample of LITMUS38i 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 <1% white colonies.

Endonuclease Activity (Nicking) - A 50 µl reaction in rCutSmart[™] Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 100 units of *NheI-HF*[®] incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

Exonuclease Activity (Radioactivity Release) - A 50 µl reaction in rCutSmart[™] Buffer containing 1 µg of a mixture of single and double-stranded [³H] *E. coli* DNA and a minimum of 200 units of *NheI-HF*[®] incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

Ligation and Recutting (Terminal Integrity) - After a 100-fold over-digestion of Lambda HindIII DNA with *NheI-HF*[®], >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*[®].

Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in rCutSmart[™] Buffer containing 1 µg of Lambda-HindIII DNA and a minimum of 200 units of *NheI-HF*[®] incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Protein Purity Assay (SDS-PAGE) - *NheI-HF*[®] is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.



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qPCR DNA Contamination (<i>E. coli</i> Genomic) - A minimum of 20 units of NheI-HF [®] is screened for the presence of <i>E. coli</i> genomic DNA using SYBR [®] Green qPCR with primers specific for the <i>E. coli</i> 16S rRNA locus. Results are quantified using a standard curve generated from purified <i>E. coli</i> genomic DNA. The measured level of <i>E. coli</i> genomic DNA contamination is ≤ 1 <i>E. coli</i> genome.

One or more products referenced in this document may be covered by a 3rd-party trademark.
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Nancy Considine

Date 07 Nov 2023

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Quality Approver

